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STRONGYLOCORIS BLANCHARD: SIX NEW SPECIES FROM NORTH AMERICA (HEMIPTERA, MIRIDAE)

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Received June 29, 1938

Only six species of Strongylocoris have heretofore been recognized from North America, but the present paper raises the total number to twelve. Eight species and certain varieties are recorded from the Palearctic region, making a total of twenty species known for the world.

This genus of Mirid bugs is composed chiefly of black, shining species, all of which have a rather similar aspect. This similarity of form has made the determination of species difficult, except when reference is made to structure of the male genitalia. The present study of the male claspers has enabled the author to separate the species without difficulty, except in the case of certain female specimens. Slight variations have been found in the number of spines on a clasper as shown for *S. stygicus* Say (Plate 1, fig. 1), but the general form of both claspers, when considered together, give distinctive characters for the separation of the species.

Strongylocoris breviatus new species

Allied to *stygicus* Say but distinguished by the yellowish first and second antennal segments, the latter with distinct black band at base; male genital claspers distinctive of the species (Plate 1, fig. 3).

Male. Length 4.3 mm., width 2 mm. Head: width 1.06 mm., vertex .56 mm. Rostrum, length 1.05 mm., just reaching to base of middle coxae, black. Antennae: segment I, length .35 mm., yellowish brown, blackish on base; II, 1.3 mm., brownish to black, brownish on basal half and black on base; III, .95 mm., blackish; IV, .47 mm., blackish. Pronotum: length .91 mm., width at base 1.51 mm. Hemelytra with costal margin moderately arcuate. Dorsum finely and closely rugulose punctate, sparsely clothed with short pale pubescence, more apparent on lateral margins of hemelytra, the paracuneus with three or four long hairs.

General coloration deep black, shining; femora black, apices yellowish, tibiae yellowish, apices and more or less on basal half of hind pair fuscous; tarsi yellowish, the apical segment black.

Female. Length 4.1 mm., width 2.2 mm.; hemelytra more sharply arcuate than in the male. Head: width 1.2 mm., vertex .64 mm. Antennae: segment I, length .36 mm., yellow, base black; II, 1.17 mm., yellow, apical one-fourth and narrow ring at base, blackish; III, .86 mm., black, yellowish at base; IV, .44 mm., blackish. Coloration of the second antennal segment distinctive of the species.

Holotype: ♂ July 2, 1926, Washington, D. C. (H. H. Knight); author's collection. *Allotype:* same data as the type. *Paratypes:* 23 ♂ ♀, taken with the type on Solidago sp. DISTRICT OF COLUMBIA—7 ♂ ♀ June 24, 3 ♂ June 30, 2 ♂ July 8, 1926, Washington (H. H. Knight). ILLINOIS ♂ 2 ♀ July 17, 1883, Anna (C. A. Hart) ♂ July 5, 1932, Antioch (Frison

et al). ♀ Aug. 25, 1925, Beech (T. H. Frison). 27 ♂ ♀ June 25, 1932, Browns, on Solidago (Ross, Dozier, Park). ♂ June 12, 1933, Bureau (Mohr and Townsend). 2 ♂ 4 ♀ July 2, 1932, Castle Rock, Grand Detour (Dozier and Mohr). ♂ June 5, 1889, Champaign (C. A. Hart). 2 ♀ June 14, 1933, Dolson (Frison and Ross). 22 ♂ ♀ June 30, 1932, Galens (Dozier and Mohr). ♂ June 22, 1932, Golconda (Ross and Dozier). ♀ June 24, 1932, Herod (Ross, Dozier, Park). ♂ May 21, 1932, Makanda (Dozier). ♂ June 14, 1933, Marshall (Frison and Ross). ♂ July 2, 1906, Mt. Carmel (C. A. Hart). 3 ♂ 2 ♀ June 14, 1930, Oakwood (Frison). ♀ July 9, 1925, Oregon (Frison). 2 ♀ May 29, 1907, Pulaski (Hart). ♂ June 25, 1932, Shawneetown (Ross and Dozier). ♂ June 9, 1885, Urbana (C. A. Hart), on Helianthus. 2 ♀ June 26, 1932, West Union (Ross and Dozier). MARYLAND—♀ July 17, 1926, Glen Echo (H. H. Knight). MASSACHUSETTS—♂ ♀ July 7, 1928, Sherborn (C. A. Frost). MAINE—2 ♀ July 10, Muddybemos; ♀ June 15, 1922, Orono (R. J. Sim). MINNESOTA—♂ July 2, 1923, St. Anthony Park; ♀ Aug. 10, 1922, Cramer; 12 ♂ ♀ Aug. 15, 1922, Isabella River, Lake Co.; 6 ♂ ♀ Aug. 20, 1920, Two Harbors (H. H. Knight). ♀ June 20, 1923, Norman Co.; 2 ♂ July 21, 1922, Minnetonka Lake; ♀ Aug. 2, 1921, Cloquet (A. A. Nichol). ♂ June 28, 1921, Hennepin Co. (A. T. Hertig). ♀ July 19, 1921, Princeton (W. E. Hoffmann). MONTANA—♂ July 23, 1912, Bozeman. NEW YORK—27 ♂ ♀ July 8, 1920, Ithaca (H. H. Knight), taken on *Solidago altissima* with nymphs. ♂ July 24, ♂ July 26, 1916, Ithaca (H. H. Knight). ♂ June 28, ♂ July 26, 1914, 2 ♂ Aug. 5, ♂ Aug. 12, ♂ Aug. 13, ♀ Aug. 19, 1915, Batavia (H. H. Knight), on Solidago. ♂ ♀ July 27, 1916, McLean (H. H. Knight). NEW HAMPSHIRE—♂ June 30, 1929, Hampton (S. A. Shaw). PENNSYLVANIA—♂ June 28, 1919, Hartstown (D. M. Delong). WYOMING—♂ Aug. 17, 1927, Ten Sleep (H. H. Knight). CANADA—ALBERTA: 9 ♂ ♀ Aug. 17, 1924, Slave Lake (O. Bryant). NOVA SCOTIA: 13 ♂ ♀ July 30, 1929, Portaupique (C. A. Frost).

Strongylocoris atritibialis new species

Distinguished from *stygicus* Say by the more arcuate hemelytra, black tibiae, and in structure of the male genital claspers.

Male. Length 4.6 mm., width 2.4 mm. Head: width 1.21 mm., vertex .65 mm. Rostrum, length 1.12 mm., reaching to base of middle coxae. Antennae: segment I, length .34 mm.; II, 1. 26 mm.; III, 1.00 mm.; IV, .43 mm.; black. Pronotum: length1.12 mm., width at base 1.77 mm. Hemelytra with costal margin strongly arcuate. Dorsal surface nearly glabrous, rugulose punctate, sparsely clothed with fine, short pubescent hairs, more evident on cuneus and embolium. General coloration deep black, membrane brownish black; legs black, tibiae and tarsi sometimes brownish black, but hind tibiae always black. Genital claspers distinctive of the species (Plate 1, fig. 6).

Female. Length 4.5 mm., width 2.5 mm.; embolar margins more strongly arcuate than in the male. Head: width 1.3 mm., vertex .78 mm. Antennae: segment I, length .35 mm.; II, 1.21 mm.; III, .95 mm.; IV, .40 mm. Pronotum: length 1.12 mm., width at base 1.86 mm. More robust than the male but very similar in coloration.

Holotype: ♂ June 23, 1920, Ithaca, New York; author's collection. *Allotype:* same data as the type. *Paratypes:* ♂ ♀, taken with the types.

COLORADO—4 ♂ 5 ♀ August 11, 1925, Ute Creek Ranch, Fort Garland (H. H. Knight). ♂ June 12, ♂ June 28, 1900, Fort Collins; 8 ♂ ♀ August 19, 1898, Dixon's Canyon, Fort Collins (E. D. Ball). CONNECTICUT—♀ June 27, 1920, Kinningworth (W. E. Britton). ♀ June 22, 1920, Orange (P. Garman). DISTRICT OF COLUMBIA—10 ♂ ♀ June 24, ♀ June 30, 2 ♀ July 2, 1926, Washington (H. H. Knight). ILLINOIS—♂ July 7, 1909, Algonquin (Nason). 8 ♂ 10 ♀ June 10, 1932, Antioch (Mohr and Townsend). 3 ♂ 8 ♀ July 5-7, 1932, Antioch (Frison et al), 2 ♀ June 12, 1936, Antioch (Ross and Burke). ♂ June 25, 1932, Browns (Ross, Dozier and Park). ♀ July 2, 1932, Castle Rock, Grand Detour (Dozier and Mohr). ♂ June 8, 1931, Charleston (Frison). 5 ♂ 2 ♀ May 17, 1932 (H. L. Dozier). ♂ May 22, 1916, ♂ 5 ♀ May 12, 1916, Dongola (C. A. Hart). ♀ June 25, 1932, Rocky Branch, Dolson (Frison and Mohr). ♂ July 6, 1917 (C. A. Hart); ♂ May 27-31, 1932 (H. L. Dozier). ♂ May 15, 1932, Fountain Bluff (Frison, Ross and Mohr). 2 ♂ 2 ♀ June 30, 1933, Galena (Dozier and Mohr). ♀ June 28, 1893, 2 ♂ July 7, 1892, Galesburg (Stromberg). ♂ June 10, 1936, Grays Lake (Ross and Burke). ♀ May 31, 1933, Havana (C. A. Mohr). ♂ May 29, 1936, Herod (Ross and Mohr). ♂ ♀ June 9, 1933, Joliet (Mohr and Townsend). 2 ♀ May, 1932, Makanda (Dozier and Mohr). ♂ May 24, 1914, Muncie (C. A. Hart). ♂ ♀ May 25, 1932, Pulaski (H. L. Dozier). ♀ May 27, 1928, Shawneetown (T. H. Frison). 2 ♀ June 4, 1932, Sheldon (Frison and Mohr). 2 ♂ June 4, 1932, St. Anne (Frison and Mord). 2 ♀ June 11, 1936 (Ross and Burke); 2 ♀ July 15, 1926 (T. H. Frison). IOWA—16 ♂ ♀ June 4, 1926, 2 ♀ June 9, 1925, 20 ♂ ♀ June 18, 1925, Ames (H. H. Knight). KENTUCKY—♀ May 12, 1921, Lexington (K. S. B.). MASSACHUSETTS—♀ July 7, 1928, Sherborn (C. A. Frost). MINNESOTA—♂ June 12, 1922, Faribault; ♂ June 25, 1921, Madison; ♂ 2 ♀ June 28, 1921, Olivia; 3 ♂ 5 ♀ July 2, 1923, St. Anthony Park (H. H. Knight). ♂ June 18, 1922 Minneapolis; ♂ June 25, 1922, Ramsey Co.; ♂ ♀ June 28, 1921, Hennepin Co. (A. T. Hertig). ♂ ♀ July 10, 1920, Morrison Co.; ♀ June 21, ♂ June 24, 1921, Owatonna (A. A. Nichol). MISSOURI—♂ 2 ♀ May 16, ♂ May 23, Glencoe; ♀ June 19, 1937, St. Louis (R. C. Froeschner). NEW JERSEY—♂ ♀ June 2, 1925, Campgaw (F. M. Schott). NEW YORK—♀ June 14, 1915, ♂ July 12, 1914, Batavia; ♀ July 7, 1920, Ithaca; ♂ June 27, 1915, Portage; ♂ July 3, 1920, McLean Bogs, Tompkins Co. (H. H. Knight). ♀ June 14, 1919, White Plains (J. R. Torre-Bueno). NORTH CAROLINA—♂ June, 1907, Hendersonville (F. Sherman). ♀ June 200, 1916, Swannanoa (R. W. Leiby). NORTH DAKOTA—♂ 2 ♀ July 14, 1920, Kidder Co. (A. A. Nichol). SOUTH DAKOTA—♀ July 29, 1927, Deadwood (H. H. Knight). ♂ ♀ June 12, 1923, Brookings; ♂ June 23, 1923, Philip (H. C. Severin). OHIO—♂ July 17, 1926, Wilmington (S. A. Watson). PENNSYLVANIA—7 ♂ ♀ June 8, 1921, 6 ♂ ♀ June 7, ♀ June 8, 1923, Chambersburg (J. R. Stear). 2 ♀ June 17, 1917, Charter Oak (J. N. Knull). TENNESSEE—♀ May 15, 1917, Knoxville, (Geo. G. Ainslie). WASHINGTON—2 ♂ 1 ♀ July 6, 1937, Dayton (R. E. Miller). WYOMING—♀ July 16, 1920, Yellowstone National Park (A. A. Nichol). 46 ♂ ♀ August 7, 1927, Shoshone National Forest (H. H. Knight). CANADA—ALBERTA: ♂ July 31, 1921, ♀ July 29, ♂ August 6, Nordegg (J. McDonnough). ONTARIO: 4 ♂ ♀ July 10, 1915, Parry Sound (H. S. Parish).

Strongylocoris pallidicornis new species

Allied to *atritibialis* as indicated by the structure of the male genital claspers but differs in the longer second antennal segment which greatly exceeds the width of head across eyes; first and second antennal segment and all three pairs of tibiae, pale.

Male. Length 4.55 mm., width 1.9 mm. Head: width 1.08 mm., vertex .56 mm. Rostrum, length 1.12 mm., reaching to middle of intermediate coxae, blackish, middle one-third yellowish. Antennae: segment I, length .34 mm., yellowish, fuscous on base; II, 1.25 mm., pale to yellowish, frequently fuscous on apical one-fifth; III, .91 mm., blackish, narrowly pale at base; IV, .47 mm., fuscous. Pronotum: length .91 mm., width at base 1.55 mm. Hemelytra with costal margin only very slightly arcuate. Dorsum sparsely clothed with moderately short, brownish pubescent hairs, more prominent on embolium and lateral margins of pronotum. General coloration black, shining, dorsum rather thickly rugulose punctate; membrane brownish black, paler apically. Legs black, apices of femora and coxae, tibiae, and tarsi except apical segment, pale to yellowish. Genital claspers rather similar to those of *atritibialis,* but terminal portion of right clasper somewhat different (fig. 5).

Female. Length 4.2 mm., width 2.2 mm.; embolar margins more arcuate than in the male, but not so strongly arcuate as in *atritibialis;* antennae and legs more broadly pale than in the male. Head: width 1.12 mm., vertex .62 mm. Antennae: segment I, length .35 mm., pale yellowish; II, 1.12 mm., pale yellowish, slightly fuscous on apex; III, .82 mm., fuscous, narrowly pale at base; IV, .39 mm., fuscous. Pronotum: length .91 mm., width at base 1.6 mm.

Holotype: ♂ August 19, 1927, Custer, South Dakota (H. H. Knight); author's collection. *Allotype:* July 27, 1927, Custer, South Dakota (H. H. Knight). *Paratypes:* 1 ♂ 1 ♀ July 27, 3 ♂ 3 ♀ August 19, 1927, Custer, South Dakota (H. H. Knight). COLORADO—3 ♂ 1 ♀ August 7, 1925, Stonewall, alt. 8,500 ft., near Trinidad (H. H. Knight). ♂ August 11, 1925, Ute Creek Ranch, Fort Garland (H. H. Knight). 12 ♂ 4 ♀ August 19, 1898, Dixon's Canyon, Fort Collins (E. D. Ball). WYOMING—3 ♂ 4 ♀ July 20, 1920, Yellowstone National Park (A. A. Nichol). NORTH DAKOTA—2 ♀ July 19, ♀ August 4, 1923, Trail Co. (A. A. Nichol). ♂ July 12, 1920, Cass Co. (A. A. Nichol). MINNESOTA—♂ June 20, 1921, New Ulm (H. H. Knight). ♂ June 21, ♀ June 25, ♂ July 20, 1923, Norman Co. (A. A. Nichol). 3 ♀ July 25, 1921, Eagle Bend (A. A. Nichol).

Strongylocoris hirtus new species

Distinguished from allied species with erect pubescence by the longer second antennal segment which exceeds width of head across eyes; tibiae and first antennal segment pale; male genital claspers distinctive (Plate 1, fig. 4).

Male. Length 4.6 mm., width 2.2 mm. Head: width 1.12 mm., vertex .56 mm. Rostrum, length 1.12 mm., just reaching to base of middle coxae; black, third segment except base and basal half of fourth segment, pale. Antennae: segment I, length .38 mm., pale, base fuscous; II, 1.43 mm., black, basal one-fifth pale; III, .86 mm., black; IV, .47 mm., black. Pronotum: length .98 mm., width at base .165 mm. Hemelytra with costal margin

moderately arcuate on distal half. Clothed with thickly set, erect, golden brown to blackish pubescence.

General coloration black, slightly shining; legs black, tibiae, tarsi except apical segment, and apices of femora, pale, tibial spines fuscous.

Female. Length 4.3 mm., width 2.4 mm. Head: width 1.12 mm., vertex .60 mm. Antennae: segment I, length .35 mm.; II, 1.21 mm., pale, apical one-fourth blackish; III, .74 mm.; IV, .43 mm. More robust than the male but very similar in color and pubescence.

Holotype: ♂ July 1, 1929, Ames, Iowa (H. H. Knight); author's collection. *Allotype:* taken with the type. *Paratypes:* 39 ♂ ♀, taken with the types on *Silphium perfoliatum* L. which is the host plant. 12 ♂ ♀ July 13, 27 ♂ ♀ July 18, 1927, 47 ♂ ♀ June 21, 1928, 8 ♂ ♀ July 11, 1929, 54 ♂ ♀ July 2, 1930, ♂ 3 ♀ June 24, 1931, Ames, Iowa (H. H. Knight), all collected on *Silphium perfoliatum.* ♂ 3 ♀ July 5, 1927, Ames Iowa (H. G. Johnston). ILLINOIS—♂ June 14, 1931, Charleston (H. H. Ross). ♂ June 5, 1932, Hardin (H. L. Dozier). 4 ♂ 1 ♀ June 14, 1930 (T. H. Frison), on *Silphium.* ♂ ♀ June 26, 1932, West Union (Ross and Dozier). KANSAS—♂ July, Topeka (Popenoe).

Strongylocoris ambrosiae new species

Allied to *hirtus* but readily distinguished by the smaller size, black antennae with shorter second segment, and structure of the male genital claspers.

Male. Length 3.7 mm., width 1.5 mm. Head: width .92 mm., vertex .48 mm. Rostrum, length .87 mm., reaching upon base of middle coxae. Antennae: segment I, length .23 mm.; II, .82 mm., not equal to width of head; III, .60 mm.; IV, .30 mm. Pronotum: length .69 mm., width at base 1.3 mm. Hemelytra with costal margin only very slightly arcuate. Dorsum thickly clothed with erect, brownish black pubescent hairs. General coloration black, moderately shining; tibiae and tips of femora pale to yellowish, tarsi fuscous to black, scarcely paler on base. Genital claspers distinctive of the species (fig. 2).

Female. Length 3.5 mm., width 1.8 mm. Head: width .99 mm., vertex .56 mm. Antennae: segment I, length .22 mm.; II, .92 mm.; III, .65 mm.; IV, .30 mm. Pronotum: length .78 mm., width at base 1.4 mm. Hemelytra with costal margin more arcuate but very similar to the male in color and pubescence.

Holotype: male, August 24, 1927, Emery, South Dakota (H. H. Knight); author's collection. *Allotype:* taken with the type. *Paratypes:* 46 ♂ ♀, taken with the types on *Ambrosia psilostachya.* IOWA—♀ May 22, 1936, Ames (G. Bleasdell). 2 ♂ May 29, 1936, Ames (H. H. Knight). TEXAS—2 ♂ 3 ♀ September 28, 1930, Amarillo (S. E. Jones). KANSAS —♀ July 6 (Popenoe); ♀ July 2, 10 ♂ ♀ August 17, ♀ August 18, Riley Co. (G. A. Dean). 4 ♂ ♀ August 31, Wallace Co.

Strongylocoris albibasis new species

Distinguished from known species by the small size and white base of vertex; male genital claspers distinctive of the species (Plate 1, fig. 8).

Male. Length 3.3 mm., width 1.6 mm. Head: width .82 mm., vertex .4 mm.; basal edge of vertex bluntly carinate, white. Rostrum, length .86

mm., extending to tip of intermediate coxae. Antennae: segment I, length .22 mm., black, tip pale; II, .90 mm., black; III, .61 mm., black; IV, .35 mm., black. Pronotum: length .69 mm., width at base 1.25 mm. Hemelytra with costal margin only slightly arcuate. Dorsum finely rugulose punctate, shining, sparsely clothed with short pale pubescence.

General coloration shining black, base of vertex and posterior margins of eye, white; legs black, tips of femora pale, tibiae pale, hind pair black, sometimes slightly paler on apical half; tarsi black, scarcely paler at base.

Female. Length 3 mm., width 1.7 mm.; hemelytra with costal margin rather strongly arcuate. Head: width .86 mm., vertex .47 mm.; base of head white as in the male. Antennae: segment I, length .21 mm.; II, .78 mm.; III, .56 mm.; IV, .30 mm. More robust than the male but very similar in color and pubescence.

Holotype: ♂ June 2, 1926, alt. 4,500 ft., Tombstone, Arizona (A. A. Nichol); author's collection. *Allotype:* same data as the type. *Paratypes:* 4 ♂ 1 ♀, taken with the types.

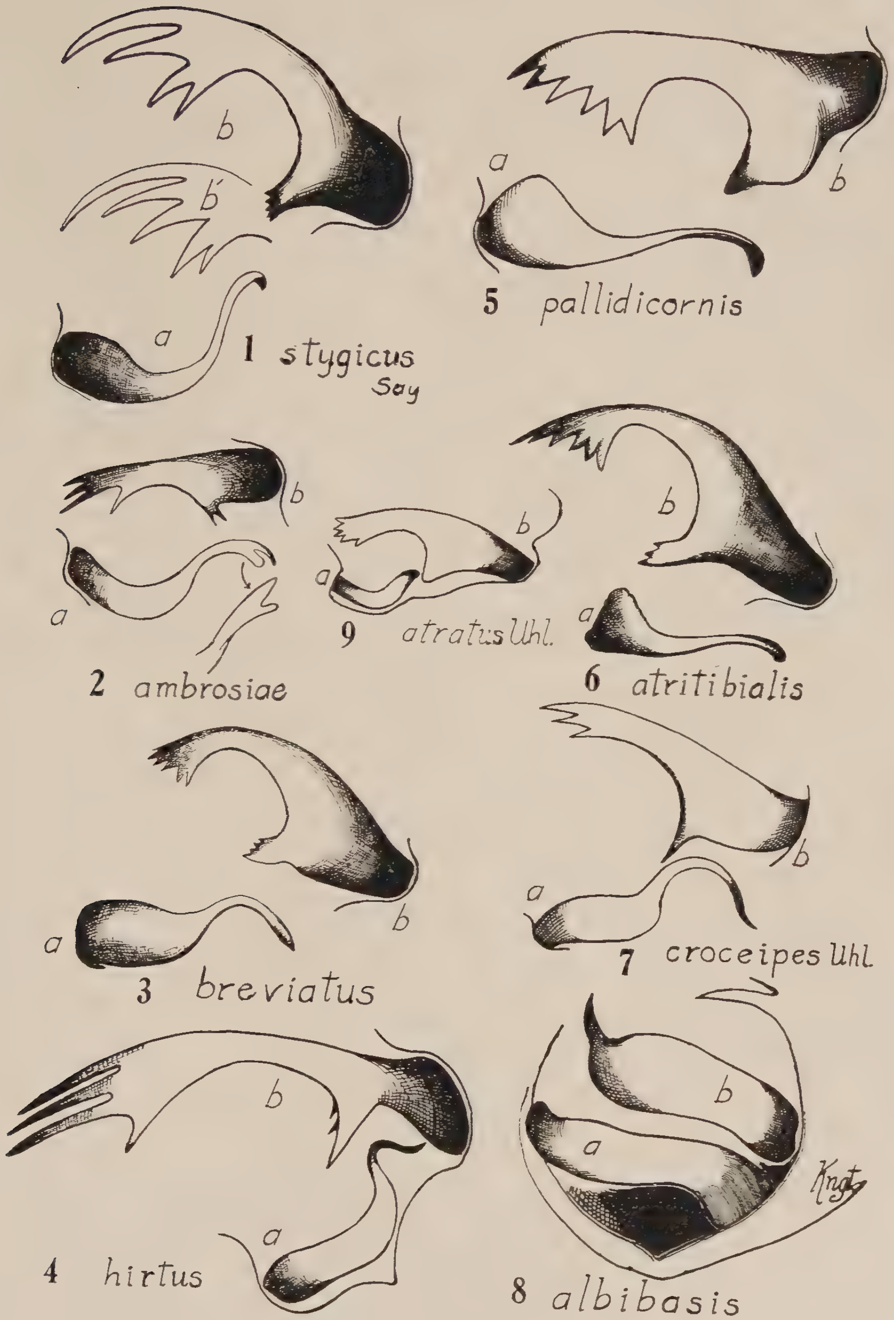
PLATE I

Explanation of figures

Male genital claspers of Strongylocoris. *a.* left clasper, postero-lateral aspect. *b.* right clasper, postero-lateral aspect. *b'*. variation in apex of right clasper.

Fig. 1. *Strongylocoris stygicus* Say.
Fig. 2. *Strongylocoris ambrosiae* n. sp.
Fig. 3. *Strongylocoris breviatus* n. sp.
Fig 4. *Strongylocoris hirtus* n. sp.
Fig. 5. *Strongylocoris pallidicornis* n. sp.
Fig. 6. *Strongylocoris atritibialis* n. sp.
Fig. 7. *Strongylocoris croceipes* Uhl.
Fig. 8. *Strongylocoris albibasis* n. sp.
Fig. 9. *Strongylocoris atratus* Uhler, drawn on reduced scale, from the type in the California Academy of Sciences.

PLATE I



1 stygicus Say
2 ambrosiae
3 breviatus
4 hirtus
5 pallidicornis
6 atritibialis
7 croceipes Uhl.
8 albibasis
9 atratus Uhl.
a
b
b'
Kngt

THE FORMATION OF VITAMINS A, B_1, AND C IN LEMNA GROWN IN THE ABSENCE OF ORGANIC MATTER

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The effect of organic material on growth and reproduction of green plants has been studied for many years. From early work on the amount of crops produced, to recent investigations on plant hormones, the subject has received constant attention, but comparatively little work has been done on the effect of organic matter on the formation of vitamins in the plant, or on conditions which may affect the quality of crops in this respect.

The question of the effect of organic matter on the vitamin content of grains was first raised in India, when an investigation was made to determine if the quantity of vitamin in plants was increased by the presence and availability of organic matter in the soil. This question is of major importance to a country in which a large proportion of the food is derived from grains, especially in view of the increasing use of inorganic fertilizers, and is of considerable interest to investigators of vitamins and of agricultural products.

McCarrison (17) in southern India found the quantity of vitamins in grains (particularly the B complex and to some extent vitamin A also) varied considerably. The variation seemed to be correlated with the organic matter available to the plant. Viswa Nath (21) came to the conclusion either that bacteria synthesized vitamins from organic matter and passed them on to the plant, or that the microorganisms produced substances which stimulated the formation of vitamins within the plant. On the other hand, at the Ohio Experimental Station, the findings of Hunt (15) seem to indicate no correlation between the presence of the vitamin and the supply of organic matter. He checked the amount of the vitamin B complex in wheat grown on plots that had been treated for 35 years with different kinds of fertilizers, and found little influence due to the treatments. A variation occurred, but it seemed to be attributable more to climate than to fertilizers or organic matter.

A somewhat similar theory to that of Viswa Nath's had been suggested by Bottomley and Mockeridge fifteen years earlier. Bottomley (4), soon after the essential nature of vitamins in animal life was recognized, introduced the idea of plant vitamins or auximones. According to this theory it was necessary to supply the organic auximones in order to obtain normal functioning of the plant, and Mockeridge (18) concluded that the more bacterial decomposition there was in organic manures, the more auximone was formed.

The claim that these organic substances were essential for the growth and reproduction of all green plants was shown by Clark and Roller (8)

[1] Part of this investigation was made possible by a grant from the Rockefeller Fluid Research Fund of Iowa State College.

to be without foundation, and this was confirmed by Saeger (19) and other investigators. Clark and Roller showed that the green plant Lemna would reproduce in inorganic solutions, in the absence of both organic matter and microorganisms. However, in some cases there were definite effects on growth and reproduction of Lemna from the addition of organic matter to the nutrient medium in which the plant was growing, especially in the presence of bacteria and other microorganisms.

Recently it has been demonstrated that plants themselves produce certain organic substances (auxins) which are essential for their life processes (16, 22). These may behave as plant hormones, being produced and transported in the plant itself, as is the case with hormones in the animal body. The auxins were at first believed to be few in number and specific in their action, but further investigation disclosed many substances which acted in a similar way (14). Further, when extracted auxins, or similar synthetic compounds, were supplied to the plant—for example, by injection, or by means of the soil or nutrient solution—increased growth or reproduction occurred, causing curvature of coleoptiles, bending of plant stems, or growth of adventitious roots.

It seemed logical that substances like the vitamins, which occurred so frequently in plants, should have some specific function in plant life. From one point of view, the value of these vitamins is that they are essential to animals, but plants would not produce them for that purpose, although animals may have evolved so as to make use of the vitamins produced. A number of the vitamins have been tested as plant growth-promoting substances and found to have a definite effect on growth. For example, van Hausen (12) reported that the addition of vitamin C to pea cultures caused an increase in dry weight, and exerted a marked influence on young plants. Bonner (3) came to the conclusion that vitamin B_1 was as necessary for plant growth as it was for the normal growth of animals.

Bonner suggests that all the various organic substances which have definite beneficial effects on plant growth or reproduction should be classed as 'plant vitamins'. This would include the auxins or plant hormones, some or all of the animal vitamins, the 'bioses' of yeast, 'pantothenic' acid and others. He also suggests that all plants may be classified as heterotrophic or autotrophic. To the first group, plant vitamins must be supplied; to the second, no organic material need be given, as the plant synthesizes its own. How many green plants are completely autotrophic is not known.

Lemna major seems to be definitely autotrophic. It has been used by one of the authors (N. A. C.) for several years in investigations on the effect of organic matter on reproduction and growth (6). This plant can produce flowers, but it usually buds asexually. It has been grown successfully, not only in soil solutions, but in entirely inorganic media, with and without microorganisms, and under artificial light, as well as in sunlight. The Lemna has passed through hundreds of generations, quite free from organic matter or bacteria, both in the media and in the plant itself. It was therefore possible to determine whether a plant so produced would contain any vitamin at all, and to make some attempt at comparison with the content of similar plants grown under more normal conditions.

VITAMIN A

Four groups of rats were given a diet deficient in vitamin A until their weights either remained stationary for several days or began to decrease markedly. Frequently the early symptoms characteristic of vitamin A deficiency were observed. After satisfactory depletion, three of the groups were fed Lemna as a supplement, while the fourth group served as a control. When the basal ration was supplemented with Lemna grown in a soil-water medium (with ample organic matter available and with no attempt to control microorganisms), the deficiency in the diet was corrected. The xerophthalmia cleared up rapidly and the rats regained health and added weight. Lemna grown in inorganic nutrient media (specially purified salt solutions, in the absence of all microorganisms), and through a long series of generations, was fed in the same way and was equally potent in meeting the deficiency. Details of these experiments were published earlier (6, 7).

VITAMIN B_1

The first attempt to find vitamin B_1 in the Lemna was made with *Drosophila melanogaster,* as the B complex had been reported essential in the life cycle of the fly, and considerably less material would be required than for rat feeding. The attempt, however, was not successful. If it had been feasible to produce the sterile larvae on synthetic media, adding the Lemna or its extract to provide the vitamin, a considerable amount of time would have been saved. To obtain a supply of Lemna, free from microorganisms and sufficient to feed groups of rats, necessitates drying and storage of the plants, and this treatment has some influence on the quantity of the vitamin, as noted in the experiments with vitamin A (7).

As the experiments with the Drosophila were unsuccessful, it was necessary to prepare the larger quantities required for rats. One lot of plants was grown in the absence of microorganisms, in the inorganic culture medium, with special precautions to exclude all organic matter. The plants were produced in Erlenmeyer flasks with cotton wool stoppers, collected once a week after checking for contamination, washed, dried and kept in the dark. These plants received light from Mazda lamps only, with the temperature held at 25°C. The non-sterile Lemna were produced in Erlenmeyer flasks or in large dishes in a soil-water mixture. These received sunlight, supplemented in winter with some artificial light. The plants were collected and dried in the same way as the others.

Two series of tests were run, about a year apart. Some vitamin B_1 was expected in the Lemna, but it was impossible to estimate how much. The first series confirmed the expectation and indicated the approximate quantity.

SERIES 1

This series included 55 rats in groups, with litter mates separated in different groups. All were fed the Chase and Sherman (5) basal ration, diet 513, without B_1, until the body weights had dropped appreciably. As a positive control, 4 rats were then fed 200 mg. each per day non-autoclaved yeast. Recovery was rapid and growth continued until shortly after the yeast was removed from the ration (fig. 1, curve 7).

The negative control groups (fig. 1, curves 1 and 1a), consisted of 12 rats. These received as much as they would eat of the basal diet and 500 mg. daily per rat of autoclaved yeast in which B_1 had been destroyed. Six of these rats died before the end of the experiment and two others shortly afterwards (fig. 1, curve 1). Each of the four remaining rats was given 50 mg. daily of the B_1 International Standard, equal to 5 International Units, and all recovered quickly (fig. 1, curve 1a). There was no doubt that B_1 was the controlling factor.

The supplements were given to each rat daily, mixed with autoclaved yeast and fed by mouth with a graduated hypodermic syringe. Groups 2, 3, and 4 (fig. 1, curves 2, 3, 4) received .25, .5, and 1 International Unit of B_1 respectively. Those on .25 unit slightly more than maintained their weights; group 3, (.5 unit), and group 4, (1 unit), produced proportionately greater gains. There was a rapid decrease in weight when the supple-

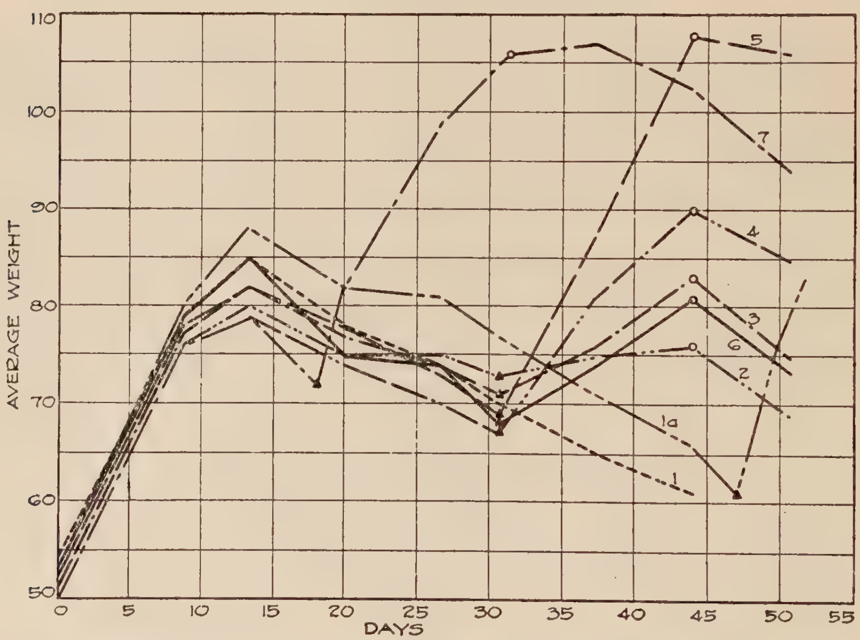

Fig. 1. Average growth curves of groups of rats fed a vitamin B_1-free basal diet, with and without supplements.

▲ Supplement started. ○ supplement discontinued.

Curve 1. Negative control group
" 1a. Negative control—late supplement 5 International Units B_1 daily per rat
" 2. .25 International Unit B_1 daily per rat
" 3. .5 International Unit B_1 daily per rat
" 4. 1 International Unit B_1 daily per rat
" 5. 500 mg. sterile, "inorganic" Lemna daily per rat
" 6. 500 mg. non-sterile, "soil" Lemna daily per rat
" 7. 200 mg. non-autoclaved yeast daily per rat

ment was discontinued (fig. 1, curves 2, 3, and 4). Curves 5 and 6, figure 1, represent the recovery of two rats, each receiving 500 mg. per day of the Lemna grown under sterile conditions in inorganic solutions, and under non-sterile conditions in the soil-water mixture, respectively. The increase in weight and the return of muscular coordination showed the presence of B_1 in both cases, and the indication was that the larger amount of B_1 was formed in the sterile Lemna which had received no organic matter.

SERIES 2

In the second series 23 rats were used. More Lemna was available, but it had been dried and stored for almost twice as long as in the first series, and some loss of the B_1 content was evident. The results of this test confirmed the preliminary experiment. The depletion ration of

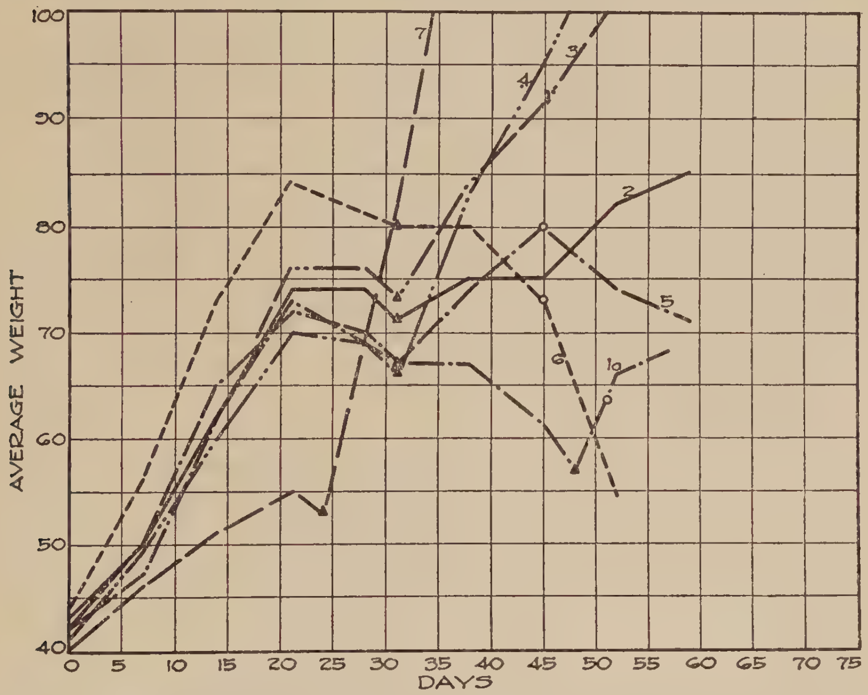

Fig. 2. Average growth curves of groups of rats fed a vitamin B_1-free basal diet, with and without supplements.

▲ Supplement started. ○ supplement discontinued.

Curve 1a. Negative control group—late supplement
" 2. .5 International Unit B_1 daily per rat
" 3. 1 International Unit B_1 daily per rat
" 4. 2 International Units B_1 daily per rat
" 5. 250 mg. sterile "inorganic" Lemna daily per rat
" 6. 500 mg. non-sterile, "soil" Lemna daily per rat
" 7. Non-autoclaved yeast

Evans, Lepkovsky and Murphy (10) was used. Each rat was given daily 500 mg. of autoclaved yeast and 50 mg. of cod liver oil, together with the supplement and a part of the basal ration. After this was consumed, free access was allowed to the basal ration.

Curve 7, figure 2, shows the positive control group and the rapid increase in weight when non-autoclaved yeast was included in the diet. The negative control group, curve 1a, at 48 days showed marked muscular incoordination, but this disappeared rapidly, and weight increased following daily supplementation with 2 International Units per rat. Groups 2, 3, and 4 received per rat .5, 1, and 2 units of the Standard Vitamin B_1 preparation, respectively. The growth curves are shown in figure 2, curves 2, 3, and 4. Group 5, consisting of 4 rats, received 250 mg. each of the dry sterile 'inorganic' Lemna; curve 5, figure 2, shows the rapid gain in average weight. The downward trend of curve 6 was arrested for a few days, following supplementation with 500 mg. of the non-sterile Lemna from the soil mixture. When the supply of this Lemna was exhausted, the rate of weight loss became greater. In this connection an interesting comparison was made with alfalfa, dried and baled in the field. Nine hundred milligrams of this was needed per day per rat before a slight increase was found in the weight of vitamin B_1-depleted rats.

The results of the two series point definitely to the fact that Lemna manufactures vitamin B_1 whether supplied with organic matter or not. In these experiments the plant produced more of the vitamin when no organic matter was supplied and when no microorganisms were present, but with the small amounts of material involved the conclusion cannot be drawn that this always would be the case. Whether the Lemna is completely autotrophic would depend upon its behavior with this and other accessory substances, and while some work has been done along this line, vitamin B_1 has not yet been tested.

VITAMIN C

Virtanen (20) grew peas in sterile culture and, from the action of vitamin C in increasing dry weight, drew the conclusion that the vitamin was a plant hormone. Van Hausen (12) found seedlings of peas, from seeds treated with vitamin C, increased their rate of growth, and Havas (13) reported similar results, but with decided variations in the effect on different plants.

In the determination of vitamin C in Lemna several difficulties were encountered in the quantitative determination. The work was done a few years ago (Frahm, 11), and no opportunity has presented itself to go further into the matter. There was no doubt, however, that the Lemna grown under sterile conditions, free of microorganisms and in the absence of all organic matter, did produce vitamin C, but whether more or less than non-sterile Lemna on soil-water mixtures was not determined.

Investigation of the vitamin C content was carried out by chemical methods. Both iodine and 2, 6-dichlorophenolindophenol were used as oxidizing agents. The plant material was extracted according to the procedure of Bessey and King (1) and Birch, Harris, and Ray (2). Both 8 per cent aqueous solutions of hot acetic acid and of trichloroacetic acid were used, and both produced colored extracts from Lemna—reddish from the 'soil' cultures and yellow from the sterile 'inorganic' cultures.

The colors were removed by the mercuric acetate treatment of Emmerie and van Eekelen (9). The reducing values averaged about four times greater for iodine than for the dye. Results, however, were irregular, although a considerable loss of the vitamin from storage was indicated.

SUMMARY

1. Lemna, grown in inorganic solutions, without organic matter, and in the absence of microorganisms, cured xerophthalmia and increased the weight of rats which had been restricted to a vitamin A-deficient diet.
2. Lemna, grown under the same conditions, produced vitamin B_1, as shown by the response of rats fed a vitamin B_1-deficient diet supplemented with the Lemna.
3. More vitamin B_1 was formed in the Lemna grown under the sterile conditions without organic matter than in the plants in a soil-water solution containing microorganisms and organic material.
4. Vitamin C was indicated in the sterile 'inorganic' Lemna by both iodine and 2, 6-dichlorophenolindophenol titrations.
5. In all three cases storage decreased the quantity of vitamin in the plants.

LITERATURE CITED

1. Bessey, O. A., and C. G. King
   1933. Jour. Biol. Chem 103: 687-698.

2. Birch, T. W., L. J. Harris, and S. N. Ray
   1933. Nature. 131: 273-274.

3. Bonner, J.
   1937. Bot. Review. 3: 616-640.

4. Bottomley, W. B.
   1917. Proc. Roy. Soc. London. 89B: 481-507.

5. Chase, E. F., and H. C. Sherman
   1931. Jour. Amer. Chem. Soc. 53: 3506-3510.

6. Clark, N. A.
   1924. Ind. Eng. Chem. 16: 249-250. 1925. Jour. Phys. Chem. 29: 935-941. 1926. Plant Physiol. 1: 273-279. 1932. Iowa State Coll. Jour. Sci. 7: 13-16. 1935. Jour. Amer. Soc. Agron. 27: 100-103.

7. Clark, N. A., and B. H. Thomas
   1934. Science. 79: 571-572.

8. Clark, N. A., and E. M. Roller
   1931. Soil Sci. 31: 299-309.

9. Emmerie, A., and M. van Eekelen
   1934. Biochem. Jour. 28: 1153-1154.

10. Evans, H. M., S. Lepkovsky, and E. A. Murphy
    1934. Jour. Biol. Chem. 107: 429-437.

11. Frahm, E. E.
    1935. Thesis, Library, Iowa State College.

12. van Hausen, S.
    1935. Nature. 136: 516.

13. Havas, L.
1935. Nature. **136**: 435.

14. Hitchcock, A. E., and P. W. Zimmerman
1935. Boyce Thompson Inst. **7**: 447–476

15 Hunt, C. H.
1927. Ohio Agr. Exp. Sta. Bul. 415.

16. Kögl, F., and H. Erxleben
1934. Zeit. Physiol. Chem. **227**: 51–73.

17. McCarrison, R.
1926. Indian Jour. Med. Res. **14**: 351-378.

18. Mockeridge, F. A.
1924. Ann. Bot. 38:723–734.

19. Saeger, *A.*
1925. Jour. Gen. Physiol. **7**:517–526.

20. Virtanen, A. I.
1936. Nature. **137**: 779-780.

21. Viswa Nath, B.
1932. Some aspects of plant nutrition. Agr. Res. Inst. Coimbatore.

22. Went, F. W.
1935. Bot. Review. **1**: 162-182.

A METHOD FOR RECORDING EVAPORATION FROM A POROUS ATMOMETER CUP[1]

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A continuous record of the amount and rate of evaporation is useful in the study of various water relations for plants and soil. Evaporation from a porous atmometer cup integrates to some extent the effects of radiation intensity and air velocity, humidity, and temperature and consequently, these cups have come into common use (4). Chalkley and Livingston (1) have described a method for automatically recording the rate of evaporation from a porous cup. Their apparatus simply records the pressure drop across a flow resistance, inserted in the line between the supply water reservoir and the porous cup. Christensen, Veihmeyer and Givan (2) have shown that at constant temperature the pressure drop across such a resistance is accurately proportional to the evaporation rate, but that appreciable corrections must be applied to compensate for viscosity changes that accompany ordinary temperature fluctuations.

Morris and Durrell (5) have devised a recording atmometer that is extremely simple to construct and gives directly the cumulative curve for the evaporation from a porous cup. With their instrument the recording pen is attached to a metal float and thus records directly the level of the water in the atmometer supply reservoir. It is difficult, however, to obtain with appreciable accuracy a record of the evaporation rate from such a curve.

The authors here describe the use of a recording drop counter for measuring the flow of water to an atmometer cup. The operating principle for this flow meter which has been described elsewhere (3, 6) consists in causing water enroute from the reservoir to the atmometer to form in drops at a dropper tip located in a kerosene filled chamber. By means of direct mechanical coupling (6) the time at which each drop falls from the dropper tip is recorded on a chronograph. These drops then pass downward through the kerosene-water interface and on to the atmometer cup.

The effects of temperature and flow rate upon drop size have already been described (3). Temperature effects are relatively small, being less than one per cent decrease in drop size for a rise in temperature of five degrees Centigrade. The dependence of drop size on flow rate is more serious and requires the use of a calibration curve. Inconvenience from this source, however, may be minimized in certain graphical treatments of the data. In a greenhouse study (7) where this type of flow gauge was used to give a continuous record of atmometer-cup evaporation-rate, the

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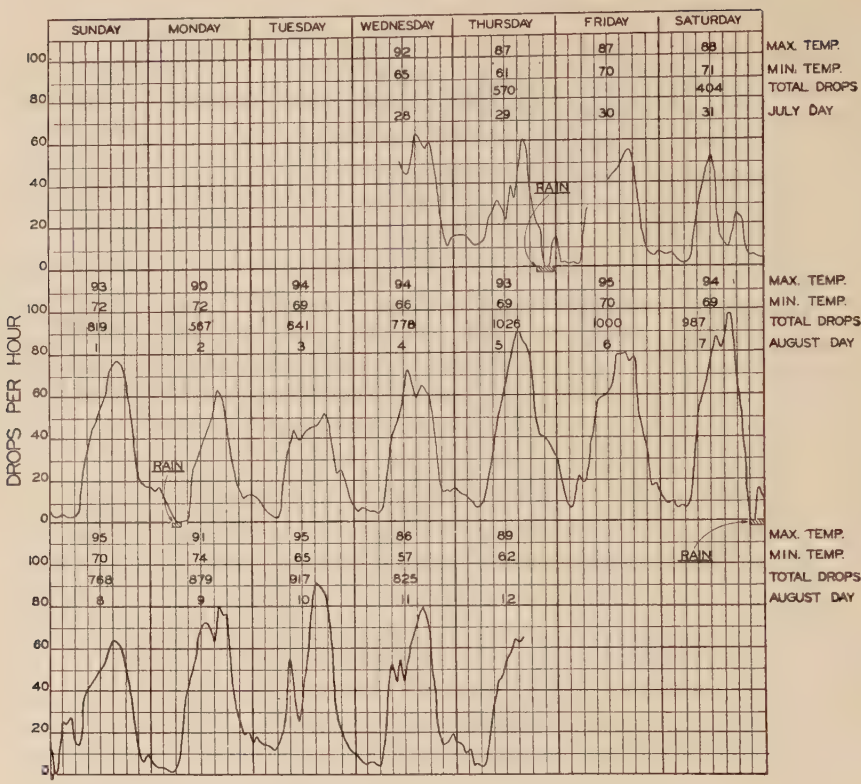
[1] Contribution from the Iowa Agricultural Experiment Station, Journal Paper No. 588. Project 553, Ames, Iowa, in cooperation with the Soil Conservation Service Experiment Station, Clarinda, Iowa.

[2] Research Associate Professor of Soils and Research Fellow, respectively, Iowa Agricultural Experiment Station, Ames, Iowa.



RATE OF EVAPORATION FROM A POROUS CELL

Fig. 1.

dropping rate was plotted against time on a linear scale. A non-linear scale based on the drop-size calibration-curve and giving evaporation rate in cubic centimeters per hour was then placed beside the dropping rate scale as an aid in interpreting the curve. For a flow rate recorder of this type the chronograph must be analyzed before either the flow rate or the cumulative curve can be plotted. It does have the advantage that both of these curves may be obtained with some precision.

Figure 1 shows a record of evaporation rate that was taken during the summer of 1937 at the Soil Conservation Service Experiment Station at Clarinda, Iowa. The porous cell used[1] was mounted one foot above a grass sod where it would have continuous exposure to the sun. Standard one-eighth inch copper tubing was used to make connection to the drop counter and water reservoir which were located in a field house approximately 20 feet from the cup. A drum speed of 20 inches per hour was used, making it possible to put a 48-hour record on one chart 20 inches long and 6 inches wide. The curves in the figure were obtained by plotting the number of drops during an hour against the time at the end of the hour. The curves

[1] The porous cell was fired from preston clay. It was light red in color and cylindrical in shape, being 5.8 cm. in diameter and 17 cm. high.

thus show the hourly average evaporation rate. No correction was made for the effect of temperature or flow rate on drop size.

For the period of record it is seen that the minimum evaporation rate usually occurred between 3 and 6 o'clock in the morning and the maximum evaporation rate usually occurred between 3 and 6 o'clock in the afternoon. Because of the many factors affecting evaporation there is no close relation between the daily maximum air temperature and maximum evaporation rate or total evaporation for the day. The graph shows that usually there occurred intermediate minima in the evaporation rate some time between sundown and midnight.

Rainfall periods as determined from recording rain gage records are shown as rectangular cross-hatched areas under the time axes in the figure. No provision was made to prevent rain water from entering the porous cell. It is interesting to note that the drop counter recorded very appreciable amounts of evaporation during periods when the recording rain gage charts indicated continuous rain. The authors know that the sharp rise in the evaporation rate which occurred during the more or less continuous four-hour rain of August 7 was occasioned by the abrupt beginning of a hard wind, because at that time they got out to repitch the tent in which they had been sleeping.

The authors plan to obtain continuous records of water loss from plants by feeding nutrient solution through a drop counter to plants growing in liquid culture in a sealed root container.

REFERENCES

(1) Chalkley, H. W., and B. E. Livingston
1929. Atmometer rates read instantaneously and an automatic continuous recorder for rate fluctuation. Ecology, **10**:37-46.

(2) Christensen, J. E., F. J. Veihmeyer, and C. V. Givan
1930. Effect of variations in temperature on the operation of the instantaneous reading atmometer. Ecology, **11**:161-169.

(3) Ivie, J. O., and L. A. Richards
1937. A meter for recording slow liquid flow. Rev. of Sci. Instr., **8**:86-89.

(4) Livingston, B. E.
1935. Atmometers of porous porcelain and paper and their use in physiological ecology. Ecology, **16**:458-472.

(5) Morris, M. S., and L. W. Durrell
1932. A recording atmometer. Ecology, **13**:100.

(6) Richards, L. A., M. B. Russell, and O.R. Neal
1937. Further developments on apparatus for field moisture studies. Soil Sci. Soc. Am. Proc., **2**:55-63.

(7) Richards, L. A., and M. B. Russell
1937. Apparatus for studying water relations in potted plants. Trans. Am. Geophysical Union, 18th Annual Meeting, 588-592.

THE HIGH FREQUENCY GLOW DISCHARGE IN HYDROGEN EXCITED THROUGH INTERNAL ELECTRODES

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From the Department of Physics, Iowa State College

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Although the alternating current discharge has been known for almost as long as the D. C. type, it is so complex that no theory has yet appeared which satisfactorily explains all the observed phenomena. For this reason, investigations of high frequency discharge characteristics have been limited usually to the experimental determination of sparking and maintenance potentials.

Two types of discharge are known as high frequency. In one, the gas is excited by the electro-magnetic field surrounding a coil carrying a high frequency current. In the other, the gas is excited by applying to it the high frequency voltage appearing across a source such as the plate coil of a vacuum tube oscillator. In this type, known as the electro-static discharge, the gas, enclosed in a glass container, may have the exciting voltage applied through external sleeve electrodes as was done by Fox and Bachman (1) or, as in this investigation, the energy may be fed directly to the gas through electrodes sealed inside the container. In the latter case, energy losses in the glass and consequent distortion of the electric field in the vicinity of the electrodes can be materially diminished.

THEORY

A theory of the glow discharge excited electrostatically has been proposed by J. Thomson (2). On the basis of a sinusoidal electric field (E), the displacement of a free electron is, according to this theory:

$$X = \frac{Ee}{2\pi v^2 m}\left(\frac{1}{2\pi}\left[\cos\phi - \cos(2\pi vt - \phi)\right] + t\sin\phi\right) \qquad (1)$$

in which ϕ is a phase angle, v is the frequency of electric field variation, and e/m is the ratio of the charge to the mass of the electron. This equation results from the resonable assumption that if the typical free electron begins to move when the electric field strength is $E\cos(-\phi)$, the velocity necessary for ionization will be most quickly attained if it is acquired in a time t_1, such that the field at this time is $E\cos(+\phi)$, for then,

$$\int_0^{t_1} \cos(2\pi vt - \phi)\,dt$$

is a maximum. From equation (1), the maximum displacement of the electron is:

$$X = \frac{Ee}{2\pi v^2 m} \qquad (2)$$

For a discharge tube with plane parallel electrodes, the equation for the sparking potential is of the form:

$$V_s = A + bpd \tag{3}$$

where A is a function of the electrodes and b is a function of the gas, p is the pressure, and d is the interelectrode distance. If V equals the potential through which a positive ion must fall to produce a certain number of electrons at the cathode surface, and V_0 be a quantity proportional to the ionization potential of the gas, the electron fall in potential V_e takes place in a distance

$$\frac{L}{4\sqrt{2}} = \frac{K}{4\sqrt{2}\,p},$$

and Thomson's theory shows

$$V_s = V_e + \frac{V_0}{K} p \left(d - \frac{K}{4\sqrt{2}\,p}\right)$$

while further analysis allows the deduction that:

$$A = V_e - \frac{V_0}{4\sqrt{2}}, \quad b = \frac{V_0}{K}$$

The analysis implies that a space charge is built up which permits the positive ions to release a sufficient number of electrons from the cathode to start the current. It also implies an abnormal fall in potential in the neighborhood of the cathode brought about by this space charge, but a uniform potential gradient throughout the remainder of the interelectrode distance d. Such implications appear reasonable in view of the difference in mobilities of the electrons and positive ions.

For moderately high frequencies of excitation, where the amplitude of oscillation is quite large, Thomson obtains for b the experimental value 18 which suggests the electron travels twelve times the kinetic theory mean free path before ionizing. This means that a smaller number of electrons can excite a discharge in the AC than in the DC type of discharge; but it also implies that, when the electronic amplitude of oscillation falls

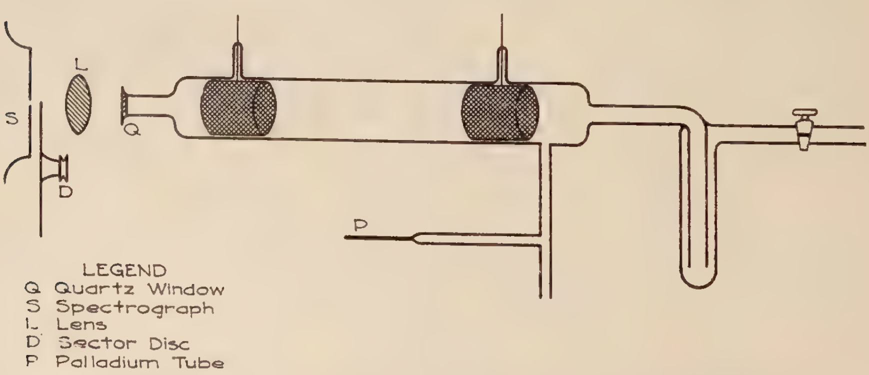

Fig. 1. Experimental arrangement of apparatus for photographing the hydrogen spectrum.

to the order of 12L (L is the mean free path of an electron), the value of the field strength necessary to produce a discharge must increase.

Although strictly speaking this theory was developed for striking potentials, it seems probable that its general features can be applied as well to maintenance potentials. This has been done in the present investigation, in which the intensity variations of certain lines in the Balmer series of hydrogen, excited by high frequency voltages applied through internal electrodes, have been studied as functions of pressure and frequency.

EXPERIMENTAL PROCEDURE

The gas system (Fig. 1) was entirely of Pyrex except for a fused quartz window, Q, connected to one end of the discharge tube by means of a graded seal, and a palladium tube, P, similarly connected to a side tube. An oil diffusion pump of the vertical stream type, charged with apiezon oil B, and a hyvac fore pump made up the evacuating system. The discharge tube, 50 cm. long and 5 cm. in diameter, was isolated from the pumping system by a liquid air trap. Especially prepared graphite electrodes, in the form of cylindrical shells 4 cm. in diameter and 4 cm. in length, were mounted in the tube 30 cm. apart. Hydrogen was admitted to the system by diffusion through palladium, the pressure being read by a Pirani guage.

The high frequency voltage was generated by a push pull oscillator powered with type 852 tubes and was measured directly by a cathode ray oscillograph. The load on the oscillator, imposed by the oscillograph, was negligible and did not affect its efficient operation. Oscillation frequencies were measured by General Radio wave meters, types 22L and 358.

Spectrograms of the discharge were made with a Bausch and Lomb medium quartz spectrograph in conjunction with a logarithmic sector disk similar to that described by Twyman and Simeon (3). The disk was constructed to the equation $-\log \theta = 0.2l$ and differed from theirs in having two symmetrically placed spirals instead of one. The value of θ represents the circumferential aperture at a distance l measured inward radially from the outermost part of the disk. The disk was driven at 3,000 r.p.m. The spectrum line lengths, photographed on Wratten and Wainwright panchromatic plates, were measured consistently to 0.1 mm. All plates were from the same emulsion batch and were developed in fresh stock solution under constant temperature and time conditions. Line intensities were reduced to unit exposure and the line lengths computed according to the equation $\log t_1 - \log t_2 = 0.2\,(l_2 - l_1)$, which means that, for constant exposure, the length of a spectrum line will vary as the intensity of that line. Spectroscopic data were obtained for various frequencies of excitation up to 5×10^7 cycles per second and for pressures from 5 to 300 microns. A peak potential of 1,500 volts was maintained between the electrodes on all runs.

RESULTS AND DISCUSSION

The results, in graphical form, (figure 2), show how the intensities of the first six lines of the Balmer series of hydrogen vary with pressure and frequency of excitation. It may be said, in general, that for each line there exists an optimum pressure and one or more favorable frequencies of excitation. The agreement between these curves and those of Fox and

and Bachman is apparent. The decrease in conductivity for wave lengths of excitation above 25 meters and below 6 meters has been recorded by Brasefield (4) and Thomson (2).

The intensity of the shorter wave length lines practically reaches that of H_α for low pressure. This suggests that the population ratios for the energy states involving these lines is closely connected with the pressure. Lowering the pressure results in longer mean free paths so that the particles attain higher velocities between collisions. Thus, their heat motions become less important compared to their field directed speeds so that the probability of excitation of the higher terms in the series approaches that of H_α.

For stable high frequency operation, sufficient positive ions must be be produced by collision of electrons with gas molecules within a few mean free paths and within a time not greater than half a cycle to fulfill the con-

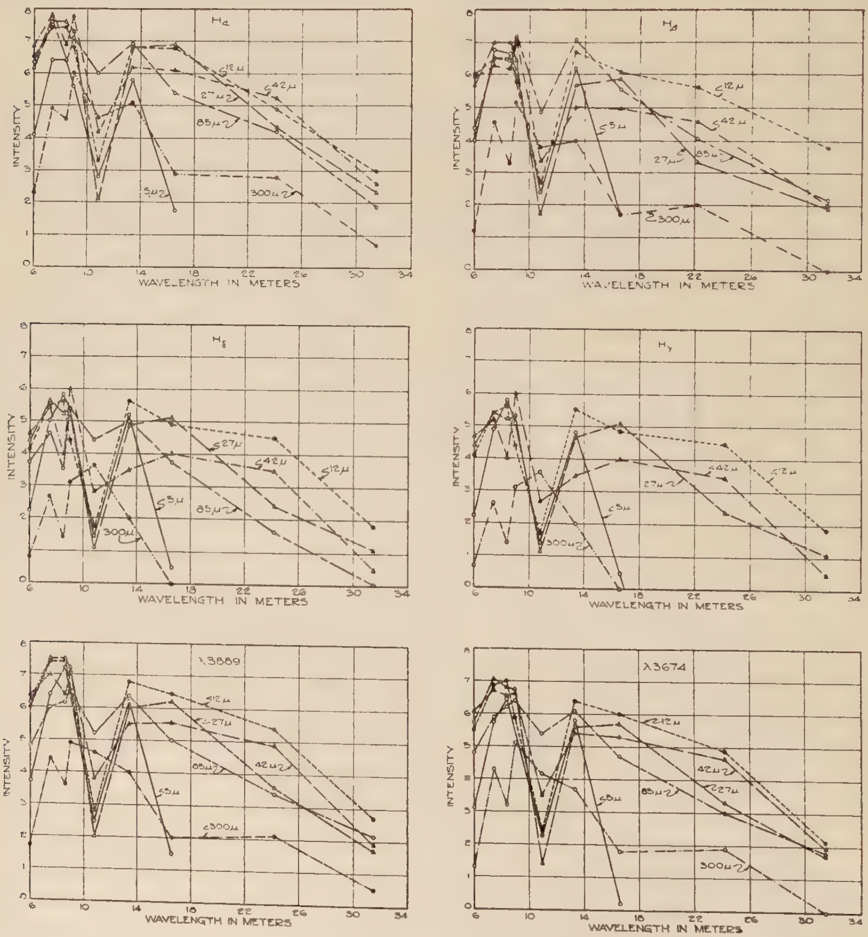

Fig. 2. Intensity vs wave length of excitation curves for different pressures for first six lines of Balmer series.

ditions for a self-maintained discharge. Obviously, at collision, the electron must have sufficient energy to ionize. When the wave length of excitation is increased, the time required for the electric force to reach its maximum value increases and hence a longer time is required for the electron to reach ionizing velocity. Thus, the efficiency of the discharge should increase with the frequency of excitation except at frequencies for which the electron oscillation amplitude falls to the order of the electron mean free path. Here the discharge efficiency would be expected to decrease.

If Thomson's Theory is applied to the present investigation, three assumptions are necessary:

1. That the electrodes are plane parallel.
2. That the cathode sheath potential gradient is of the order of 325 volts per centimeter.
3. That conditions for minimum sparking potential are the same as for discharge maintenance with maximum efficiency.

Because of the large interelectrode distance, the error introduced by the first assumption is negligible. The second is in accord with numerous probe studies made by Langmuir in direct current discharges, while Brasefield (4) and others have shown that a discharge has its maximum conductivity under the same conditions that give minimum striking potential.

A simple calculation indicates that, at the optimum pressure (30 microns), the kinetic theory mean free path is that of the order of 2.58 cm., so that, according to the discharge theory outlined, the electronic mean free path is 31 cm. (12L). If these values are substituted in equation (2), the optimum frequency of excitation is 5.5×10^7 cycles per second (wave length 5.4 meters). The experimental curves suggest a minimum in this region. Resonance phenomena of some sort probably explain the minimum at 10.8 meters shown in the curves.

The relation of the interelectrode distance and electron amplitudes of oscillation for excitation are: 2d, 8d, and 16d for the maxima; and 1d, 4.5d, and 9d for the minima (5).

The electronic mean free path for the optimum pressure is of the same order of magnitude as the electrode separation for:

$$p = \frac{12K}{d} = \frac{12 \times 7.74 \times 10^{-2}}{30} = 33 \text{ microns.}$$

These results indicate a close relation between the pressure, the tube dimensions, and the excitation frequency for maintenance of a high frequency discharge. Any variation from optimum conditions tends to increase or decrease the intensity of the spectrum line depending on whether it was originally a maximum or minimum. If the dimensions of the tube were changed, it would be expected the optimum maintenance conditions would change also, which indicates that Townsend's law of similitude is valid for this type of discharge.

REFERENCES

1. Fox, G. W., and C. H. Bachman
1936. Line intensity variations in the hydrogen high frequency glow discharge. Phys. Rev. 50: 939–942.

2. THOMSON, JOHN
1937. Sparking potentials at ultra high frequencies. Phil Mag. Series 7. 23:1-24.

3. TWYMAN, F., and F. SIMEON
1929. The logarithmic sector wedge and its use in quantitative spectrum analysis. Trans. Optical Soc. London, 31:169-181.

4. BRASEFIELD, C. J.
1930. The conductivity of a high frequency discharge in hydrogen. Phys. Rev. 35:1073-1079.

5. BURDINE, N. T.
1937. High frequency glow discharge with internal electrodes. Unpublished Thesis, Library, Iowa State College, p. 34.

LOWER COST DIETS FOR HATCHERY TROUT

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From the Iowa State Conservation Commission

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One of the major projects in the fish management plan of the Iowa State Conservation Commission is the production and stocking of trout at least seven inches long, the minimum legal length. In fact, the trout stocked in Iowa streams range from eight to ten inches in length. With such a trout program feeding costs are ever before the hatchery manager.

Prices of beef heart, a favored food item, have ranged from \$.08 to \$.125 per pound in the past few years. Certain commercial trout foods have been as high as \$.065 per pound. These prices indicate that in the production of trout for a reasonable stocking program, foods of lower prices must be sought.

Many trout feeding experiments have been made by skilled fisheries workers to determine the kind of food which is most readily taken and which would produce best growth results economically under optimum conditions.

The writer (unpublished manuscript) obtained with starved fingerling yellow pike-perch *(Stizostedion vitreum)* results that were not comparable with those noted by Titcomb and associates (1928) on starved trout which readily recovered from periods of starvation. No doubt anatomical differences in the type of stomachs of different fish play an important role not only in type of foods elected but in ability to recover from periods of starvation.

McCay and associates (1931) indicated in studies covering nutritional requirements of trout that certain diets must be balanced and that protein content of feed must be kept within certain limits.

McCay and Tunison (1934) worked out growth rate curves with brook trout *(Salvelinus fontinalis)* which showed that varying percentages of proteins and fats in several diets definitely affected the growth rate of trout.

Fielder and Samson (1935) stated that bones of canned carp might be beneficial as food particles; but this was not verified by the following described experiment.

In an attempt to find less expensive food for trout that would give satisfactory growth results, two experiments in feeding trout were inaugurated by the writer in 1936. In the first experiment, designated by "A", 3,000 brook trout were fed. In the second experiment, designated by "B", 450 brown trout *(Salmo fario)* were used. As the Iowa State Conservation Commission maintains a trout hatchery at the Backbone State Park near Strawberry Point, the feeding work was carried on there. The feeding, weighing and recording of data was personally handled by R. B. Cooper, Fish Culturist, in charge of the Backbone Station.

A comparative study was made of the values of (1) beef heart, (2) beef hearts, 50 per cent, and ground cooked carp and buffalo, 50 per cent, and (3) ground cooked carp and buffalo as food for the trout. The beef

hearts were trimmed and ground fresh. The carp and buffalo were ground entire, cooked and packed in seven-pound cans. Buffalo too small to have a market value made up 80 per cent of the ground fish and the other 20 per cent was carp of four to five pounds in individual weight.

As beef hearts composed the major portion of the food used regularly at the Backbone Station, one group of fish was fed that diet for purposes of checking the results of the other two diets.

By using the mixed diet of ground fish and beef hearts it was expected that data might be secured on a cheaper food than beef hearts alone and might illustrate a fair diet with little or no serious check on growth increments. Furthermore, the diet of ground cooked fish alone might demonstrate an inexpensive food for a given period of time and at the same instance furnish an outlet for rough fish disposal.

By using two different ages and sizes of trout it was thought data might be obtained on the practicability of mixed foods at different growth periods.

The food was weighed daily and equal volumes by weight were placed in each feeding trough. The amounts of feed were normal feeding

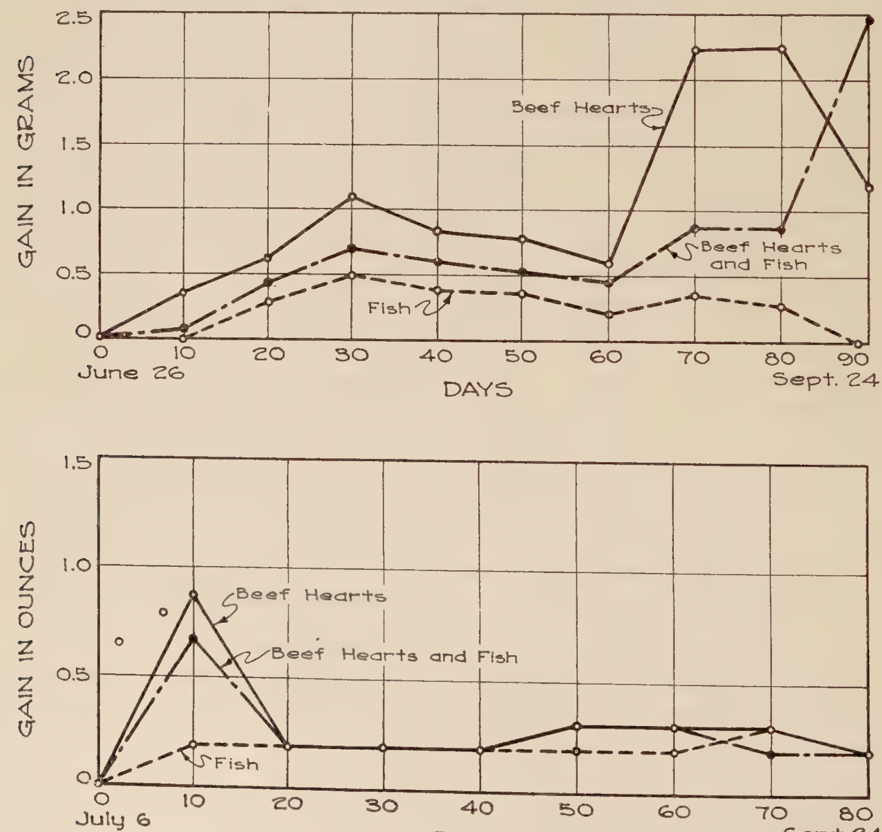

Fig. 1, A, Gain in weight of young brook trout fed on various diets; B, gain in weight of brown trout fed on various diets.

portions, and the increases in food weight were made on the fish-weighing dates.

In Experiment "A" the fish were weighed at regular ten-day intervals. Three troughs were used with 1,000 brook trout in each trough. These fish were uniform in size, about six months old, and all in a normal and healthy condition when the experiment was started. The water temperature, 50 degrees Fahrenheit, remained constant throughout the experimental period. Variables such as acidity, temperature, and oxygen content of the water were eliminated easily as a deep natural spring furnished the water supply, which was piped from the emerging point directly to the hatchery troughs. Because the temperature remained so uniformly constant, it was assumed that no marked change occurred in the oxygen content. The pH of this water supply ranged from 7.3 to 7.4 over a yearly period.

The Iowa State Department of Agriculture made a commercial feed analysis of the canned fish which showed this product to have a protein content of 17.51 per cent, fat 11.20 per cent, ash 5.20 per cent, moisture 65.00 per cent, nitrogen-free extract 1.09 per cent, and fiber none. The cost of the canned fish was $.035 per pound in seven-pound (No. 10 size) cans.

Experiment "A" was started June 6, 1936, and was discontinued September 26, 1938. The data are given in tables 1, 2 and 3. The fish weight in grams was obtained in the following manner. At intervals of 10 days five or six random groups of 100 trout were taken from each trough and weighed separately. The average of these weights for 100 fish was multiplied by 10 for each diet group, and thus the fish weight in grams in each case was computed for 1,000 trout, although some fish were lost during the 90-day period. The weight gain in grams was found by subtracting the fish weight in grams of a given weighing day from that obtained on the next date of weighing. Gain per fish in grams came from dividing the weight gain in grams for a given 10-day interval by 1,000. From the beef heart diet group 57 trout were lost, from the heart and fish diet 160, and from the cooked fish diet 464.

Attention is called to figure 1, A of Experiment "A". On all three diets, for the first 30 days the growth rate shows an almost steady daily

TABLE 1. *Experiment "A". Diet of beef hearts*

Date	Interval in days	Food weight in ounces	Fish weight in grams	Weight gain in grams	Gain per fish in grams
6-26	0	0	1,500	0	.00
7-6	10	44	1,860	360	.36
7-16	10	60	2,540	680	.68
7-26	10	80	3,660	1,120	1.12
8-5	10	80	4,480	820	.82
8-15	10	80	5,380	900	.90
8-25	10	122	6,000	620	.62
9-4	10	140	8,250	2,250	2.25
9-14	10	140	10,500	2,250	2.25
9-24	10	140	11,700	1,200	1.20

TABLE 2. *Experiment "A". Diet of beef hearts and cooked fish*

Date	Interval in days	Food weight in ounces	Fish weight in grams	Weight gain in grams	Gain per fish in grams
6-26	0	0	1,500	0	.00
7-6	10	44	1,580	80	.08
7-16	10	60	2,020	440	.44
7-26	10	80	2,720	700	.70
8-5	10	80	3,330	610	.61
8-15	10	80	3,870	540	.54
8-25	10	122	4,310	440	.44
9-4	10	140	5,190	880	.88
9-14	10	140	6,060	870	.87
9-24	10	140	8,460	2,400	2.40

increase except on the canned fish diet. After that period another 30-day interval shows a definite decrease in gain, and then again a sharp 10-day increase in gain, which levelled off for another 10-day period and then made a sharp decrease in the two diets of beef and fish alone, but showed a remarkable increase in the combined beef and fish diet in the last 10 days. Just why there was such a variation in gain shown by the combination diet in comparison with the diets of beef alone and fish alone during these last 10 days of the experiment can hardly be conjectured since the diets paralleled each other in results up to this period. An explanation might be advanced that at this period the mixed diet was most suitable for nine-months-old fish and points to a cyclic diet demand. The marked fluctuations in growth in each individual trough parallel and definitely point out relative merits of the respective diets at the given ages. The results demonstrate that further study needs to be made over a longer period of time, at least 18 months, the normal age of trout at stocking time. Such a study would give better information on both determinate and indeterminate growth rates.

To the culturist feeding the fish, no sudden changes in fish failing to feed or exhibiting unusual hunger were apparent at any time on the

TABLE 3. *Experiment "A". Diet of cooked fish*

Date	Interval in days	Food weight in ounces	Fish weight in grams	Weight gain in grams	Gain per fish in grams
6-26	0	0	1,500	0	.00
7-6	10	44	1,540	40	.04
7-16	10	60	1,830	290	.29
7-26	10	80	2,350	520	.52
8-5	10	80	2,730	380	.38
8-15	10	80	3,090	360	.36
8-25	10	140	3,300	210	.21
9-4	10	140	3,640	340	.34
9-14	10	140	3,960	320	.32
9-24	10	140	3,890	—70	—.07

TABLE 4. *Experiment "B". Diet of beef hearts*

Date of Weighing	Interval in days	Food in ounces	No. of fish	Food weight in ounces	Gain in ounces	Gain per fish in ounces
7-6	0	0	150	270	0	.0
7-16	10	120	150	405	135	.9
7-26	10	120	150	435	30	.2
8-5	10	120	150	465	30	.2
8-15	10	120	150	495	30	.2
8-25	10	120	150	540	45	.3
9-4	10	120	150	585	45	.3
9-14	10	120	150	630	45	.3
9-24	10	120	150	660	30	.2

beef heart diet. The fish fed on beef hearts were in excellent condition throughout the experiment. The trout in the beef heart-cooked fish trough were not as deeply colored or quite as uniform in size at the end of the feeding experiment as those fed on beef hearts alone. The fish fed on the cooked fish alone became very uneven in size and in poor physical condition as the work continued.

The early unevenness in size became more marked as time passed and mortality increased with regularity. It was apparent that the bone particles, although soft enough for food, were unpalatable. It also appeared that young trout after striking food particles several times desisted, perhaps through fatigue, and therefore failed to continue feeding until nutritional requirements were appeased. Mortality among the fish on the cooked fish diet became so heavy in 90 days that Experiment "A" was stopped.

In Experiment "B", larger fish were used in out-door raceways. In this experiment three troughs were set up with 150 brown trout in each retainer. The following tables give feeding details and results.

In making an analysis of this experiment, figure 1, B indicates a very comparable gain after the first 20 days, and bears out other feeding experiments being conducted that better results are obtained with cooked

TABLE 5. *Experiment "B". Diet of beef and cooked fish*

Date of Weighing	Interval in days	Food in ounces	No. of fish	Food weight in ounces	Gain in ounces	Gain per fish in ounces
7-6	0	0	150	270	0	.0
7-16	10	120	150	375	105	.7
7-26	10	120	150	405	30	.2
8-5	10	120	150	435	30	.2
8-15	10	120	150	465	30	.2
8-25	10	120	150	510	45	.3
9-4	10	120	150	555	45	.3
9-14	10	120	150	585	30	.2
9-25	10	120	150	615	30	.2

TABLE 6. *Experiment "B". Diet of cooked fish*

Date of Weighing	Interval in days	Food in ounces	No. of fish	Food weight in ounces	Gain in ounces	Gain per fish in ounces
7-6	0	0	150	270	0	.0
7-16	10	120	150	300	30	.2
7-26	10	120	150	300	30	.2
8-5	10	120	150	330	30	.2
8-15	10	120	150	360	30	.2
8-25	10	120	150	390	30	.2
9-4	10	120	150	420	30	.2
9-14	10	120	150	465	45	.3
9-24	10	120	150	495	30	.2

fish if fed to the larger sized fish. The sharp gain the first ten days is no doubt due to the fact that these fish had less competition for food after being removed from the holding pond.

It is believed that the percentage of non-nutritious food particles, such as pieces of bone and scales, of the cooked fish, when fed to very young fish is one factor that might create an uneven growth in the early stages of the feeding experiment and might be a condition of inanition.

In making conclusions on comparative value and cost of the different foods the analysis of Experiment "B" is shown in table 7.

SUMMARY

On a cost basis, ground cooked carp and buffalo was the most economical of the three diets. However, the quality and gross pounds of fish produced is an important factor, which makes a decision necessary as to which is most desired—quality or quantity—for money expended.

The experiment demonstrated that fingerling fish can be fed on a mixed diet of beef hearts and canned fish at a saving on food cost of 28 per cent and on canned fish alone at a saving of 40 per cent without serious impairment of quality. In Experiment "A" this was negatively shown with smaller fish.

Growth rate studies point to physiological phases affecting fish cultural results as seen in ability of trout fed on canned fish to recover from prolonged feeding on a diet that is apparently lacking in some important nutritional factor.

TABLE 7. *Analysis of Experiment "B"*

Diets	Pounds	Cost per Pound	Fish Pounds	Cost per pound of trout produced
Beef Hearts	60	$.10	24.375	$.25
Beef hearts and cooked fish	60	.0675	21.5625	.18
Cooked fish	60	.035	14.0625	.15

Both experiments show relative values of the different diets on growth rate and weight gain for a definite period.

LITERATURE CITED

FIEDLER, R. H., and V. J. SAMSON
1935. Survey of fish hatchery foods and feeding practices. Memorandum No. 2526, Bureau of Fisheries, U. S. Department of Commerce, Washington, D. C.

MCCAY, C. M., A. TUNISON, MARY CROWELL, D. K. TRESSLER, S. P. MACDONALD, JOHN W. TITCOMB, and EBEN W. COBB
1931. The nutritional requirements of trout and chemical composition of the entire trout body. Transactions of the American Fisheries Society, **61**:58-79.

MCCAY, C. M., and A. V. TUNISON
1934. Report of the experimental work of the Cortland Hatchery, New York State Conservation Department.

TITCOMB, J. W., EBEN W. COBB, MARY F. CROWELL, and C. M. MCCAY
1928. The nutritional requirements and growth rates of brook trout. Transactions of American Fisheries Society, **58**:205-226.

CONTENTS

ABSTRACTS OF DOCTORAL THESES

Note: Complete copies of these theses may be consulted at the Library, Iowa State College, Ames, Iowa.

THE ELECTROMETRIC DETERMINATION OF THE SOLUBILITY OF SOME HYDROXIDES[1]

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Received June 25, 1938

The present problem developed in an attempt to learn whether magnesium forms complexes with ammonium salts in dilute solutions.

Time of flow against concentration curves were made for solutions containing a fixed amount of magnesium chloride and varying amounts of ammonium chloride. These curves were found to resemble the same curve for solutions containing potassium chloride alone in varying concentrations, except that the minimum was reached at a lower concentration of the ammonium salt. This shifting of the minimum may be explained by the fact that the chloride ion, from the magnesium chloride, represses the ionization of the ammonium chloride. As no maximum was noted, through the ratio of one mole of magnesium chloride to two moles of ammonium chloride, it was concluded that no complex was formed.

The curve obtained by plotting refractometer readings for magnesium chloride-ammonium chloride mixtures, similar to those above, against concentrations of ammonium chloride gave a straight line. No conclusions, however, could be drawn from these results as mercuric chloride and ammonium chloride mixtures also gave a straight line and these mixtures were reported by Shibata, (5) from his work on absorption spectar, as containing a complex.

The work of Shibata, (5) on the absorption spectra of $HgCl_2$-NH_4Cl mixtures was duplicated, in a somewhat abbreviated manner, in this laboratory. Using an iron spark, observations were made upon the absorption spectra of O.1 M $HgCl_2$, of O.1 M NH_4Cl, and of a mixture of the two solutions in equal proportions. These observations were made in the region between 2300 and 3000 Å. The shifting of the absorption ends toward the longer wave lengths was very striking. The end of the absorption band of the mixture extended much more into this region than that of either of the solutions alone.

Observations were made, in the same region, upon one molar magnesium chloride, upon one molar ammonium chloride, and upon a mixture of the two solutions in equal proportions. No difference in the absorption spectra was noted. The absorption ends, however, had been shifted entirely out of this region by the hydrochloric acid which had been added to prevent the hydrolysis of the magnesium salt. Inavailability of the proper apparatus prevented this study being continued in the shorter wave length regions.

As the preceeding work gave results from which no well established conclusion could be reached electrometric titrations of $MgCl_2$, by means of NaOH and by means of NH_4OH, were resorted to. The scheme used by Britton (1) of projecting the almost straight parts of the curve as straight lines and then taking the intersection of these two projections

[1] Original thesis submitted August, 1937. Doctoral thesis number 446.

as the point at which precipitation began, was used. The concentration of the hydroxyl ion was calculated from the potential and the concentration of the magnesium ion was calculated from the equivalents of base added to start the precipitation. The solubility products calculated from these data, agreed in order of magnitude with each other and with the generally accepted value. The results from the titration of 0.025 M $MgCl_2$ with 0.1 N NaOH gave a solubility product of 2.19×10^{-11}. Seven titrations of 0.05 M $MgCl_2$ with 0.25 N NH_4OH gave solubility products varying from 1.7×10^{-11} to 4×10^{-11}. These results justify the conclusion, that no complexes are formed by magnesium and ammonium salts in the dilute solutions studied.

An attempt was made to determine the solubility product of magnesium hydroxide by the electrometric titration of magnesium hydroxide with ammonium chloride, by noting the potential and the total volume at which the precipitate completely dissolved. Due to the difficulty encountered in accurately determining this point of complete solution, the results obtained by this method were not so satisfactory. The results from six deetrminations varied from 1.1×10^{-11} to 9.2×10^{-11}.

A further attempt to determine the solubility product of magnesium hydroxide was made, by determining the pH of a saturated solution of the hydroxide. The solubility product varied more widely in this method, values from 2.45×10^{-12} to 8.3×10^{-11} being obtained. The pH of the solution varied with the method of preparation of the hydroxide. Part of this variance was due, no doubt, to the precipitation of basic salts rather than the pure hydroxide. Solutions prepared by saturating water with magnesium oxide consistently gave results higher than those prepared from the precipitated hyroxide. These higher values check somewhat closer the generally accepted value of 3.4×10^{-11}.

Although this method gave less consistent results than either of the other methods, it was thought to be worth while to investigate it using other hydroxides as the procedure was much simpler. Using this method, values of 1.115, 1.147 and 1.141 in grams per liter were obtained for solubility of calcium oxide at 25°C. These values compare favorably with the value of 1.148 grams per liter of water calculated from Leyson and Moody's (3) data at the same temperature. The solubility, at 25°C, of thorium hydroxide prepared by saturating a solution with thorium dioxide was found to be 2.4×10^{-5} g. per liter. This value checks in order of magnitude with that of 2×10^{-5} reported by Spitzin (7) for the same temperature. Lanthanum calculated as the sesquioxide gave 0.008 g. at 25°C per liter as compared to 0.004 g. at 29°C reported in the Handbook of Chemistry and Physics (2). The original source of this data on Lanthanum could not be found. The solubility product of zinc hydroxide deetrmined in this manner was found to be $1.69 \times {}^{-21}$ which agrees with Britton's (1) value of 10^{-21} but differs considerably from the generally accepted value of 1.8×10^{-14}. The solubility of stronium oxide was found to be 0.561 g. to 0.644 g. per 100 ml by this method. The pH of the solutions giving these values varied by 0.06. From the results given above it can be seen that this method is useful only in determining the order of solubility as a small difference in pH makes a large difference in the calculated value for the solubility.

The solubility of strontium oxide as determined varied so much from Sidersky's (6) value of 0.82 g. per 100 grams at 25°C that it was

decided to check the solubility by ordinary analytical methods. The solubility was determined by the ordinary volumetric method, by precipitation as strontium sulphate and by an evaporation method in which the strontium hydroxide solution was treated with an excess of six normal sulphuric acid, and then evaporated to dryness and ignited over a Bunsen flame. Some difficulty was encountered in obtaining checks closer than two or three parts per thousand, by the three methods, on individual samples. The mean values, however, from a number of determinations checked satisfactorily. Eighteen determinations by the evaporation method gave a mean of 0.8547, twenty-eight determinations by precipitation gave a mean of 0.8552 and fifteen volumetric determinations gave a mean of 0.8544. An approximate mean from the three methods is 0.855 g. per 100 ml or 0.850 g. per 100 grams. This value is much closer to Riedel's (4) value of 0.849 g. per 100 ml than to Sidersky's (6).

SUMMARY

1. The viscosity data, although their interpretation is somewhat questionable, offer fair evidence that no complex is formed by magnesium and ammonium salts in dilute solution.

2. The refractive indices of solutions were found to be unsuitable as evidence of the nonformation of complexes.

3. The absorption spectra although somewhat limited in scope gave no evidence of complex formation between magnesium and ammonium salts. This study is worthy of further work.

4. Agreement between the electrometric titration of magnesium chloride using sodium hydroxide and the same titration using ammonium hydroxide offers strong evidence that no complex is formed in the latter case.

5. The electrometric titration of magnesium hydroxide using ammonium chloride is not very satisfactory due to difficulty in observing accurately the disappearance of the precipitate.

6. The determination of the solubility of magnesium oxide, thorium dioxide, lanthanum hydroxide, calcium oxide and strontium oxide through the determination of the pH's of the saturated solution was found to give results agreeing in order of magnitude with the generally accepted values. The results on zinc oxide were somewhat questionable.

7. The solubility of strontium oxide at 25°C is established as 0.855 g. per 100 ml.

LITERATURE CITED

1. Britton, H. T. S.
1925. J. Chem. Soc. 127:2110-2159; 1932. Hydrogen Ions. pp. 295-231. Chapman and Hall, Ltd., London.

2. Hodgman, C. D.
1935. Handbook of Chemistry and Physics. p. 372. Chemical Rubber Publishing Co., Cleveland.

3. Leyson, L. T., and G. T. Moody
1903. J. Chem. Soc. 93:1767-1775.

4. Riedel R.
1906. Z. physik. Chem. 56:250.

5. Shibata, Y.
1922. Japan J. Chem. **1**, 1-18.

6. Sidersky, D.
1921. Bull. assoc. chim. sucr. dist. **39**, 167-177.

7. Spitzin, V. I.
1917. J. Russ. Chem. Soc. **49**, 357-370. Original not seen. Abstracted in C. A. **17**, 3291 (1923).

METALATION OF CYCLIC COMPOUNDS[1]

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Metalation has been defined as the reaction wherein a hydrogen attached to a carbon atom is replaced by a metal to form a true organometallic compound[2]. The agent used to bring about this reaction might be a free metal, an inorganic compound, or an organometallic compound. A survey of representative examples of metalation was presented, with particular emphasis upon the course of the reaction and the conditions under which it took place. The investigation of examples of metalation was undertaken in order to correlate details of the present knowledge and to add further information regarding the positions involved and the yields obtained in the metalation of various compounds.

Since different solvents were to be employed for the metalations, some attention was given to the preparation of organometallic compounds in various media. *n*-Butyllithium was prepared from *n*-butyl chloride and lithium in benzene, pentane, hexane, butyl ether, and dioxane. The yield of *n*-butyllithium in each case as determined by titration represented approximately 65-70 per cent of the theoretical value. Tri-*n*-butylamine, however, proved unsatisfactory as a solvent for the same preparation. The preparation of phenylsodium, phenylpotassium, and phenylenedisodium was investigated in various solvents.

Dibenzothiophene was subjected to the action of *n*-butyllithium in ethyl ether, benzene (at 80°), pentane (at 35°), hexane (at 65°), dioxane (at 30°), butyl ether (at 30° and 80°). The yield of metalation product was negligible in all solvents but ethyl and butyl ether. When dibenzothiophene was treated with *n*-butyllithium in butyl ether at 30° for twenty hours, a 61 per cent yield of 4-dibenzothiophenecarboxylic acid was obtained on treatment with carbon dioxide, while the same reaction, when heated to 80° for twenty hours, gave a 90 per cent yield of the same acid. When dibenzothiophene was treated with *n*-amylsodium prepared in petroleum ether according to the directions of Morton[3], a 37 per cent yield of 4-dibenzothiophenecarboxylic acid was obtained.

The metalation of bibenzyl was studied using as metalating agents *n*-butyllithium in ether, *n*-butylsodium in tributylamine, and *n*-butylpotassium in benzene. After the reaction products had been carbonated, the acids were isolated and identified. Metalation with *n*-butylsodium and *n*-butylpotassium yielded α, β-diphenylsuccinic acid (30 per cent and 52 per cent, respectively). *n*-Butyllithium, however, gave a poor yield (0-1 per cent) of *p*-(β-phenylethyl)-benzoic acid.

To study the metalation of substituted methane derivatives, phenyl-*p*-tolylmethane and phenyl-α-naphthylmethane were treated with *n*-butyllithium for thirty-six and twenty-four hours, respectively. Carbonation formed 49 per cent of phenyl-*p*-tolylacetic acid and 80 per cent of phenyl-

[1] Original thesis submitted June, 1938. Doctoral thesis number 478.
[2] Gilman and Young, *J. Am. Chem. Soc.*, **56**:1415. 1934.
[3] Morton and Hechenbleikner, *J. Am. Chem. Soc.*, **58**:2599. 1936.

α-naphthylacetic acid. Observing the increasing yield of substituted acetic acids obtained from these compounds, it was suggested that a group of similarly substituted derivatives of methane would be a satisfactory series to study to determine the effect of variations in a portion of a molecule upon the extent of metalation.

To continue the investigation of compounds containing an active methylene group, 9,10-dihydroanthracene was treated with an ether solution of *n*-butyllithium for twenty hours giving, on carbonation, 80 per cent of 9,10-dihydro-9-anthracenecarboxylic acid and 8 per cent of 9,10-dihydro-9,10-anthracenedicarboxylic acid.

When biphenyl was treated with *n*-butyllithium in ether, the yield of acidic material represented 7-15 per cent of the theoretical value, being identified as a mixture of *o*-and *p*-phenylbenzoic acids. In benzene, however, *n*-butyllithium did not cause the metalation of the nucleus. Methyllithium in butyl ether proved valueless due to the rapid cleavage of the ether, while the low yield (5 per cent) of acid obtained after *n*-butylsodium had reacted upon biphenyl in petroleum ether indicated the inapplicability of that solvent for metalations.

In the metalation of naphthalene by *n*-butyllithium, a mixture of α- and β-naphthoic acids was obtained. The amount of acidic material varied with the time of heating the reaction, amounting to 13 per cent in fifteen hours and 20 per cent in thirty hours. Phenylcalcium iodide did not cause metalation of the nucleus. *n*-Butylsodium in tributylamine yielded 28 per cent of the acid mixture after thirty-six hours of refluxing.

In an attempted metalation of phenanthrene by *n*-butyllithium, the trace of acid obtained was identified as 9-fluorenecarboxylic acid. The probable source of this acid was the fluorene present as an impurity in the starting material[4]. The phenanthrene nucleus did not undergo metalation.

Furan and 2-methylfuran were treated with phenyllithium to yield, after carbonation, 38 per cent and 17 per cent of furoic and 5-methyl-2-furoic acid, respectively. 2,5-Dimethylfuran, under the same conditions, gave an unstable acid which evolved carbon dioxide upon acidification.

Since all compounds containing an ether linkage had undergone metalation by organoalkali compounds only in the position ortho to the ether bridge, more ethers were investigated in a search for a broad generalization. As a result of the metalation of ethers, it was suggested that the metalation of compounds containing an ether linkage might serve as a method for the introduction of substituents into the position ortho to the oxygen bridge.

2-Methoxydibenzofuran was metalated by *n*-butyllithium to yield, after carbonation, 60 per cent of a mixture of 2-methoxydibenzofurancarboxylic acids. The mixture contained approximately three parts of 2-methoxy-1-dibenzofurancarboxylic acid to one part of 2-methoxy-3-dibenzofurancarboxylic acid.

After treatment with *n*-butyllithium and subsequent carbonation, anisole yielded 19 per cent of *o*-methoxybenzoic acid and 40 per cent of di-*o*-anisyl ketone. The high yield of ketone demonstrated the disadvantage of employing carbon dioxide as a reagent for the study of the products of a metalation involving an organolithium compound. When phenyl-

[4] Jeanes and Adams, *J Am. Chem. Soc.*, **59**:2608. 1937.

sodium in benzene was employed, 44 per cent of the same acid was obtained after 24 hours and 64 per cent after 48 hours.

The metalation of phenyl ether and its analogs (phenyl sulfide and phenyl selenide) was effected with *n*-butyllithium. After six hours, 54 per cent of *o*-phenoxybenzoic acid and 24 per cent of *o*-phenylmercaptobenzoic acid were obtained. When the time was increased to twenty hours, yields of 60 per cent of *o*-phenoxybenzoic acid and 30 per cent of *o*-phenylmercaptobenzoic acid showed that an increase in metalation occurred on increased time of reaction. Some cleavage of phenyl sulfide was evident from the odor of thiophenol, which was present only after the longer period of heating. When phenylsodium, suspended in benzene, served as the metalating agent, 56 per cent of *o*-phenylmercaptobenzoic acid was obtained. The odor of thiophenol was practically absent in this run. Phenyl selenide did not give a metalation product but was cleaved to butyl phenyl selenide and benzoic acid, which indicated the course of reaction as:

$$C_6H_5SeC_6H_5 + C_4H_9Li \rightarrow C_6H_5SeC_4H_9 + C_6H_5Li \xrightarrow{CO_2} C_6H_5CO_2H$$

It was predicted that the cleavage of phenyl telluride would be still greater. Possibly this tendency of compounds to display increasing instability toward metalating agents with decreasing tendency to undergo metalation might be extended from Group VI to other groups of the periodic table.

Ethynylsodium in liquid ammonia was treated with 1-heptyne and phenylacetylene. Reaction occurred in both cases to form heptynylsodium and phenylethynylsodium, respectively. The organometallic compound was identified by adding benzophenone and isolating the corresponding carbinol. In regard to the relative acidity of the three compounds, they lie in the following order, with the hydrogen in phenylacetylene probably more acidic than that of 1-heptyne.

$$\left.\begin{array}{r} C_5H_{11}C \equiv CH \\ C_6H_5C \equiv CH \end{array}\right\} > HC \equiv CH$$

In an effort to correlate acidity with metalation, the radicals attached to hydrogen were placed upon a parabolic curve symmetrical to the positive vertical axis so that reading along the vertical axis from top to bottom showed the decreasing tendency of the compounds to undergo metalation.

The radicals were assigned positions upon the curve such that any radical when attached to hydrogen (RH) would react with the organometallic compound (R′M) of any radical below it on either side of the parabola.

$$RH + R'M \rightleftarrows R'H + RM$$

By means of this curve, it may be possible to predict yields in the metalation of compounds from a knowledge of the relative lability of the radical attached to the acidic hydrogen, or *vice versa*, to predict relative lability from the yields of metalation products.

THE ECOLOGY AND MANAGEMENT OF THE BLUE-WINGED TEAL (QUERQUEDULA DISCORS (LINNAEUS))[1]

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The purpose of this investigation was to correlate the life habits and requirements of the Blue-winged Teal [*Querquedula discors* (Linnaeus)] with the effects of its present agricultural environment in the hope of developing a more substantial niche for the bird in Iowa. The research was initiated July 1, 1932 and continued until February 1, 1937. The greater part of the observations were made in Clay, Palo Alto, Dickinson, and Emmet Counties, Iowa. Supplementary observations were made in Minnesota, Nebraska, South Dakota, North Dakota, Manitoba, and Mexico.

The Blue-winged Teal is known by at least 47 vernacular names over its breeding, migratory, and wintering ranges. The sex ratio of 5,090 birds averaged 59 per cent males and 41 per cent females.

The breeding range of the bird was segregated into five types. They are named as follows in the order of their importance: true prairie area; mixed prairie area; boreal forest area; deciduous forest area; and lake forest area.

Fall migration began each year in Iowa about August 15 and usually was completed by November 1. The major portion of the flight occurred in the Mississippi Valley and Central Flyways. Shooting seasons in the northern states added little if any impetus to the fall migration.

The wintering grounds of the teal extend over thousands of square miles in Mexico, Central America, South America, and the southern part of the United States. It was determined that at least 95 per cent of the Blue-winged Teal winter south of the southern border of the United States. The southernmost record for the bird is Ovalle, Chile (Schalow, 1898). The birds were shot for sport and market purposes on the wintering grounds observed in Mexico.

Early in January from the wintering grounds the birds started northward on the spring flight. With few exceptions, the flyways over which they passed were those used during the fall migration. The main flight passed through the northern states between April 1 and April 30 each year.

Courtship began on the wintering grounds late in December and early January. Courtship became evident about the same time that the nuptial plumage of males became apparent and when the birds were getting started on the spring flight. By the end of March, when the first birds reached Iowa, the process of courtship had become a daily affair. The climax of courtship and mating was reached just prior to the nesting season.

The average distance of nests from water was 41.5 yards. It was found that 95.6 per cent of the nests were located within 220 yards of the shore-

[1] Original thesis submitted June 25, 1937. Doctoral thesis number 422.

line. The average elevation of nests above the marsh level was 2.4 feet. The average number of eggs found in 341 nests was 9.3 per nest.

The ducklings hatched 21 to 23 days after incubation began. So far as could be determined the young were led by the mother to water within twelve hours after hatching.

Of 223 nests, 40.4 per cent were destroyed by adverse weather conditions, agricultural practices, predators, and other causes. Only 14.8 per cent of twenty-seven discovered renesting attempts were successful. The number of young that reached the migratory stage was 5.1 per successful nesting female.

Bluegrass *(Poa pratensis)*, the most common type of nesting cover, constituted the cover of 160, 47 per cent, nests. One hundred and thirty-two nests, 38.8 per cent, were found in slough grass *(Spartina Michauxiana)*. Forty-eight nests, 14 per cent, were found in alfalfa *(Medicago sativa)*. The bluegrass and slough grass often had other grasses and forbs mixed in with them.

The presence of ducklings in the respective types of rearing cover combinations indicated the value of such plant associations for rearing habitats. The rearing plant combinations are listed as follows in the order of their importance: great bulrush *(Scirpus validus)*, round bulrush *(S. occidentalis)*, and river bulrush *(S. fluviatilis)* associes; river bulrush, great bulrush, and bur-reed *(Sparganium eurycarpum)* associes; river bulrush and cat-tail *(Typha latifolia)* associes; river bulrush and sedge *(Carex riparia)* associes; reed *(Phragmites communis)* and cat-tail associes; and, bur-reed, sweet flag *(Acorus Calamus)*, and larger blue flag *(Iris versicolor)* associes.

Approximately 75 per cent of the food found in 358 stomachs consisted of plant material. The four plant families, Cyperaceae, Najadaceae, Gramineae, and Polygonaceae were by far the most common groups of plants represented in the stomachs. Animal food constituted about 25 per cent of the food eaten by the Blue-winged Teal. Of the invertebrate foods eaten, insects and molluscs out-numbered other forms. The vertebrates were represented by fishes found in five stomachs.

The proper amount of grazing by cattle on marsh margins to insure permanent pasture is conducive to some teal nesting. Many areas, particularly inlets to water areas, should be revegetated to curtain soil erosion and in so doing would provide hay for live stock and nesting cover for teal, pheasants, and other wildlife.

The prairie habitat seemed to furnish the best breeding conditions for the Blue-winged Teal. The timbered tracts had but little nesting cover for teal and in addition supported a number of animals that are known to be predators of nesting ducks and their young. Not one successful nest was found in cover beneath trees or shrubs.

It was found that 95.6 per cent of the puddle ducks nested within 220 yards of the shoreline of a marsh, slough, or pot-hole regardless of the size of such a water area. Thus, the small water areas as a whole were conducive to greater duck production.

By sampling a lake region and obtaining data concerning nest densities, nest destruction, successful nests, and juvenile survival a fairly accurate census can be made to determine the year's production.

The development of appreciation of our wildlife resources the past few years in the United States seems to lead toward a permanent pro-

gram for the perpetuation and increase of many wildlife species. Correct land use practices, water conservation, soil conservation, and the economic evaluation of sporting and non-sporting species have all aided and will continue to better the environment for the Blue-winged Teal.

LITERATURE CITED

SCHALOW, HERMAN
1898. Die Vögel der Sammlung Plate. Zool. Jahrb., Supplementband. 4:641-749 (Fauna Chilensis, v. 1).

A CRITICAL STUDY OF THE PRECISION AND VALUE OF HALOGEN ADDITION REACTIONS APPLIED TO DAIRY RESEARCH[1]

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In order to determine the importance of the quantity of free unsaturated fatty acids in butter fat, a precise semi-micro iodine number method was needed. When this work was initiated (1932), no semi-micro iodine number method had been described in the literature. It seemed logical, therefore, that a standard method should be adapted for use in this study.

Preliminary results indicated that the reaction of the Hanus (1) reagent was especially sensitive to variations in sample weight. Hübl iodine numbers calculated with the Schmidt-Nielsen and Owe (4) formula, did not depend greatly upon the weight of sample but the age and acidity of the reagent were important factors.

The following halogenating reagents (prepared according to the directions given in the literature cited) were studied on a semi-micro scale for butter fat and insoluble butter acids: Hübl (4), Wijs (1), Hanus (1), Rosenmund and Kuhnhenn (3) and Kaufmann (2). Iodine number determinations were made with each of these reagents on a sample of butter fat and the insoluble acids obtained from it at varying reaction periods up to one week. This study also included the Hübl reagent in which 95 per cent ethyl alcohol was replaced by absolute methyl alcohol. The reactions were carried out in the dark at 25°C. ± 0.4.

Iodine numbers were plotted against reaction periods for each of the above reagents. If equilibria were attained, there should be flat sections (zero slopes) in the curves. The curve obtained with the Kaufmann reagent and butter fat showed but little slope from 30 to 168 hours and with the insoluble acids from 70 to 168 hours. The Hübl reagent gave similar curves when the values were calculated according to the Schmidt-Nielsen and Owe formula, but when the average of the beginning and final blanks was used for the iodine number calculations, no flat sections in the curves were evident. Both the Kaufmann and Hübl reagents gave higher values for the fat than for the acids; this anomalous behavior can not be explained satisfactorily.

None of the other curves seemed to show any indication towards a zero slope when plotted on such a scale as to include reaction periods up to one week, but the initial values of fat and acids were in a reasonable relationship to each other. When the portions of these graphs representing the reaction on butter fat from 0 to 12 hours were plotted on a much larger scale, the Kaufmann, Hanus and Rosenmund and Kuhnhenn curves approached zero slopes for variable periods of reaction. The Wijs and Hanus curves were similar although the slopes were somewhat greater with the Wijs reagent.

The iodine numbers obtained with the Rosenmund and Kuhnhenn

[1] Original thesis submitted March, 1938. Doctoral thesis number 456.

reagent on butter fat increased only about 0.3 unit from 30 min. reaction period to 5 hours. None of the other methods gave values as nearly constant as these over a reaction period of this length. It was considered that these results indicated that further study was warranted with the Rosenmund and Kuhnhenn reagent.

As a result of this study the following conditions were worked out for its use: Weigh from 10 to 100 mg. of butter fat or insoluble butter acids into a 125 ml. iodine flask and dissolve in 2 ml. of carbon tetrachloride. Add 5 ml. of 0.1N pyridine sulfate dibromide in acetic acid freed of reducing compounds, and close the flask with a glass stopper on the surface of which one drop of syrupy phosphoric acid was spread to prevent escape of reagents. Rotate the contents of the flask a few times and allow the reaction to take place in the dark at 25° C. for 4 hours. After the reaction period, add 2 ml. of 10 per cent potassium iodide to the rim of the flask, cautiously turn the stopper in order that small gas bubbles will go through the potassium iodide. Thoroughly mix the potassium iodide wtih the reactants and then add 25 ml. of oxygen-free water and mix well. Titrate the iodine with sodium thiosulfate (approximately 0.011N) to the complete disappearance of the yellow color (a slight excess of thiosulfate). Stopper the flask and shake violently, rinse liquid adhering to stopper into the flask and add 4 to 5 ml. of 0.5 per cent starch indicator. Back titrate the excess of sodium thiosulfate with iodine in potassium iodide (approximately 0.005N) from a burette graduated to 0.02 ml.

When sample weights are approximately alike, the semi-micro Rosenmund and Kuhnhenn method yields replicate determinations which agree within 0.3 of an iodine unit.

It was considered that as final checks on the semi-micro Rosenmund and Kuhnhenn method the following factors should be studied: 1. effect of excess reagent on the iodine number obtained for butter fat and its insoluble acids, 2. agreement of replicate determinations on weighed samples, 3. comparison of the value by this method with the value obtained with a carbon tetrachloride solution of iodine monobromide conducted in such fashion that substitution should be detected and 4. comparison of the value by this method with that obtained by the bromine vapor method.

It is considered from these studies that excess reagent from 14 to 90 per cent will cause a variation of 1.0 unit in the iodine number of butter fat, while variation of excess reagent from 20 to 90 per cent will cause a variation of approximately 0.5 unit with the insoluble acids from butter fat.

Replicate determinations on weighed samples should check within 0.5 unit.

The attempted determination of a "true" iodine number by measuring substitution and correcting the iodine number for it did not prove satisfactory nor did it afford the desired comparison.

The comparison with the bromine vapor method which should give values very closely representing only the degree of unsaturation of butter fat indicate that for this fat the Rosenmund and Kuhnhenn method, used as described above, gives very nearly the correct iodine number.

The bromine vapor method was studied with regard to its adaptation for use with butter fat and its acids. Further study is necessary to determine the precision of this method.

LITERATURE CITED

1. Association of Official Agricultural Chemists
1936. Official and tentative methods of analysis, p. 410-412. 4th ed. Washington, D. C.

2. Kaufmann, H. P.
1935. Studien auf dem Fettgebiet. p. 23-24. Maurer and Dimmick, Berlin.

3. Rosenmund, K. W., and W. Kuhnhenn
1923. Eine neue Methode zur Jodzahlbestimmung in Fetten und Ölen unter Verwendung von Pyridinsulfatdibromid. Z. Nahr. Genussm. 46:154-159.

4. Schmidt-Nielsen, S., and A. W. Owe
1923. Die Bestimmung der Jodzahl. I. Vergleichende Untersuchungen über die Jodzahl der Fette. Skrifter Videnskapsselskapet. I. Kristiania. Mat. natur. Klasse. No. 15.

SOME FACTORS AFFECTING THE TIME OF SOLUTION OF DEXTROSE[1]

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Dextrose[2] is quite soluble even in cold water, however, the time required to prepare solutions within five per cent of a saturated solution at a given temperature is too long for the practical preparation of syrups. Because of the increased consumption of purified dextrose as a constituent in food preparations the problem of solubility time has become an important one. It was the purpose of the present investigation to determine the more important factors affecting the solution time of dextrose.

Sandera[3] has summarized various methods for determining the solution time of sugars, no one method of which is applicable in commercial practices. A method was developed, therefore, which was adaptable to large scale preparations. The apparatus used consisted of a series of Soxhlet extraction flasks, each with a separate stirring unit, arranged in a black lined constant temperature bath controlled by a rheostat sensitive to 0.05°C. The sample being tested for solution time was placed in one of the flasks and stirred at the desired temperature until solution was complete. By means of a strong light source directed into the flask against the black background it was possible to check duplicate runs within two per cent even when the method was adapted to runs using one hundred pounds of dextrose. In order to obtain consistent results it was necessary to prevent settling out of sugar crystals by control of the stirring rate, shape of container, size of dextrose crystals and rate of addition of the dextrose.

At all concentrations the solution time of hydrated dextrose increased regularly with a temperature decrease, whereas in the case of anhydrous dextrose a pronounced increase in solution time was observed at definite temperatures and concentrations. In general the time for complete solution of anhydrous dextrose was ten minutes or less when the percentage concentration was not more than one-half the numerical value of the temperature in Fahrenheit degrees.

Initial concentrations of anhydrous dextrose were ten to fifteen per cent higher than those of the hydrated form. Rapid crystallization of hydrated dextrose occurred in the more concentrated initial solutions of anhydrous dextrose, thus proving the higher solubility of the anhydrous form as well as the rapid conversion of anhydrous to hydrated dextrose in solution. Conversion of hydrated to anhydrous dextrose is a slow reaction as exemplified by the slow solubility of the hydrated form.

$$\text{Anhydrous dextrose} + H_2O \underset{\text{slow}}{\overset{\text{rapid}}{\rightleftharpoons}} \text{hydrated dextrose}$$

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[1] Original thesis submitted March, 1938. Doctoral thesis number 460.

[2] In this abstract the term "dextrose" refers to the alpha-form of 4-glucose except where otherwise stated.

[3] Sandera, Z. Zuckerind. Czechoslovak. Rep., **61**:275. 1937.

The equilibrium mixture after dextrose hydrate crystallization consisted of approximately fifteen per cent anhydrous and eighty-five per cent hydrated dextrose.

A number of compounds yielding basic solutions when dissolved in water were tested for their catalytic effect upon the dissolution of dextrose. The pH values for these solutions, both before and after adding the dextrose, were determined by means of a Coleman electrometer having a glass electrode.

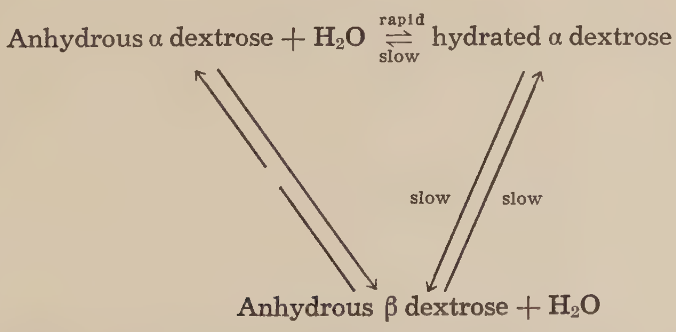
In solutions of the alkaline hydroxides, including LiOH, NaOH, KOH, $Ca(OH)_2$, $Sr(OH)_2$ and $Ba(OH)_2$, the solution time of dextrose was a direct function of pH or normality of reagent solution. Time of solution, in other reagents, however, was not determined by pH, but was dependent upon the nature of the components of the reagent solution. There was no difference in the catalytic effect among the metallic ions in any salt series.

Approximately twice the catalytic effect was shown by the sodium or potassium salt of maleic acid as by that of its geometric isomer, fumaric acid. Aqueous solutions of organic bases such as pyridine or quinoline had little influence on the solution time of dextrose. The methyl and ethyl substituted ammonium hydroxides decreased in their catalytic effect as the weight of the molecule was increased. Soluble sodium silicates were effective in direct proportion to the sodium oxide-silicon oxide ratio.

In a buffered mixture, such as the solution resulting from the titration of a phosphoric acid solution with sodium or potassium hydroxide, a maximum solution time resulted at a pH of approximately 3.8.

Catalysts of mutarotation were, in general, found to be correspondingly efficient as solution catalysts. Reagents which catalyzed mutarotation to the extent that equilibrium between the alpha- and beta-forms was established within fifteen minutes caused the alpha-dextrose to dissolve within the same period of time. Beta-dextrose as well as mixtures of alpha- and beta-dextrose containing as much as seventy-five per cent of the alpha-form dissolved almost instantaneously, whereas an equal concentration of the alpha-form alone required three hours for complete solution.

In accordance with the data presented the following equilibrium depicts the condition during the dissolution of dextrose:



Any agent or reagent which tends to produce a more soluble form or condition of dextrose will accelerate its solution.

HIGH FREQUENCY GLOW DISCHARGE WITH INTERNAL ELECTRODES[1]

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High frequency electric discharges in hydrogen were excited by voltages applied to a discharge tube by means of internal carbon electrodes. The intensities of certain lines in the hydrogen spectrum were studied quantitatively as a function of frequency of excitation and pressure.

The high frequency potential was supplied by a push-pull oscillator which used 100 watt No. 852-type tubes. The potential difference applied between the electrodes was measured by a cathode ray oscillograph and maintained at a constant peak potential of 1500 volts.

The gas system was made entirely of pyrex except for a quartz window connected by a graded seal to one end of the discharge tube and a palladium tube simililarly connected to a side tube. An oil diffusion pump that was air-cooled and of the vertical stream type charged with Apeizon oil B which was heated electrically and a Hyvac pump made up of the evacuating system. A liquid air trap was used to condense all impurities that could be frozen out. The pressure was measured by a pirani gauge. Hydrogen was admitted to the discharge tube by diffusion through palladium.

The discharge tube was 50 cm. long and 4.8 cm. in diameter, and each end was drawn to 2.5 cm. by means of shoulder seals. The tube was baked-out for several hours while on the pumps in an especially built electric furnace at a temperature between 450° and 500°C. Several times during the process the tube was flushed with hydrogen.

Spectrograms of the discharge were taken with a Bausch and Lomb medium quartz spectrograph in conjunction with a logarithmic sector disc. This type of disc gives spectral lines whose lengths are proportional to their intensity. W. and W. panchromatic plates of the same emulsion batch were used and were developed with developer D-19 from the same stock solution under the same conditions of temperature and time.

The data were obtained for pressures from 5 to 300 microns and for frequencies of excitation from 0 to $5 \cdot 10^7$ C. P. S. Intensity vs. wavelength curves were plotted for different pressures. An explanation of the maxima and minima which occur in the curves and the optimum pressure was given on the basis of J. Thomson's (2) theory of sparking potentials.

The results of the investigation show:

1. There is a variation in intensity of the spectrum lines for different pressures and frequencies of excitation.

2. The results are very similar when either internal or external electrodes are used.

[1] Original thesis submitted July, 1937. Doctoral thesis number 442.

3. The ratios of the intensities of H_β, H_γ and H_δ to H_α increase for low pressures.

4. The ratios of the populations in the higher order states increase with a decrease in pressure.

5. For low pressures the probability of excitation is as great for the higher frequency terms as for the lower ones.

6. Thomson's theory for sparking potentials applies to maintenance potentials.

7. The optimum pressure is such that approximately 12 times the kinetic theory mean free path of the electron is equal to the interelectrode distance.

8. The amplitudes of oscillation of the free electron for wavelengths which give line intensity minima and maxima are multiples of the electronic mean free path.

9. The impurities which are commonly observed in this type of discharge phenomena were eliminated by the design of the tube and the use of liquid air.

10. The most striking observation is the similarity of the intensity vs. wavelength curves to those obtained by Bachman (1).

BIBLIOGRAPHY

1. Bachman, Charles
1935. Effect of high frequency excitation upon intensities of spectral lines. Unpublished Thesis. Library, Iowa State College, Ames, Iowa.

2. Thomson, John
1937. Sparking potentials at ultra-high frequencies. Phil. Mag. ser. 7. 23:1-24.

STUDIES ON CHLORINE DISINFECTION[1]

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Chlorine compounds having disinfectant properties have been used for over a century and their consumption today probably surpasses that of all other disinfectants combined. They are employed as sterilizers, for instance, in the maintenance of safe water supplies, in medical practice, in many food industries, in the chlorination of sewage and sewage disposal-plant effluents, and in the control of slime formation in pulp and paper mills.

Originally used in the form of sodium and calcium hypochlorites, chlorine has more recently been employed in the form of chlorine gas in water solution, and in various types of compounds generally known as "chloramines," which possess an :N-Cl linkage.

The methods of estimating the strength of chlorine disinfectants are the same as those employed in analyzing hypochlorite bleaching solutions. The original term "available chlorine" has been retained although there are objections to its use.

A review of the literature reveals a lack of agreement in regard to the relative germicidal power of chlorine compounds and the manner in which they exert germicidal action. It was therefore decided to investigate various aspects of the chlorination process, particularly the relative germicidal activity of the several forms of chlorine compounds, and to note the effect of alterations in reaction (pH), temperature and concentration upon disinfection efficiency.

Bacterial spores were found to be particularly suitable and applicable for the objectives of this study. The culture employed had been used previously in studies on disinfection with alkalies and the organism, *Bacillus metiens* (nov. sp.), was further described. A dried spore suspension in powdered lactose was employed. No appreciable changes in numbers of viable cells per unit weight were observed and the spores did not exhibit detectable changes in resistance to chloramine-T during storage, for a period of over 6 months. Those observations suggested that the dried spores were not undergoing any physiological changes. The dried spores, when suspended in distilled water and connected to a Barcroft-Warburg manometer, did not reveal any evidence of respiration. Spore suspensions in distilled water showed no appreciable diminution in numbers after several weeks exposure at room temperature.

The germicidal efficiencies of chlorine disinfectants were found to be markedly affected by temperature, concentration, and reaction (pH) alterations. The results of the investigation are of interest in that they give useful information regarding the relative germicidal power of chlorine compounds and they account for certain of the conflicting opinions previously reported. The germicidal activity of chloramine-T solutions was found to be affected as follows:

[1] Original Thesis submitted December, 1933. Doctoral thesis number 260.

(a). The reaction (pH) of the disinfecting solution markedly influenced the killing time, the germicical efficiency increasing with increasing acidities. For example, a solution with 2,000 p.p.m. available chlorine, at an initial reaction of pH 8.7, gave a killing time of 64 hrs., whereas at pH 6.0 the killing time was only 5.4 hrs.

(b). Doubling the concentration resulted in a reduction of the killing time to approximately one-half.

(c). Rises of 10° C. in temperature (in the range 25° to 55° C.) resulted in a reduction of the killing time by approximately 71 per cent when employing solutions with an initial reaction of pH 8.7. The same temperature change with solutions at pH 6.0 resulted in killing-time reductions of about 82 per cent. These temperature effects were approximately uniform up to 55° C. for a given solution and reaction.

Concerning the disinfecting action of hypochlorites it was found that:

(a). The reaction (pH) markedly affected germicidal properties which were increased as the acidity rose. For example, with 1,000 p.p.m. available chlorine at a reaction of pH 11.3, the killing time was 64 min., whereas at pH 7.3 the killing time was less than 20 sec.

(b) In solutions of two commercial calcium-hypochlorite preparations employed, the "available chlorine" was not a measure of the germicidal power (even with various concentrations of the same preparation). For example, the killing times obtained with 1,000 and 100 p.p.m. available chlorine of the same compound were not appreciably different. This apparently anomalous result is explained by the fact that the more dilute solution was less alkaline (due to dilution of the stabilizing agent) with resultant increased germicidal power which was found to be approximately proportional to the calculated amount of hypochlorous acid present.

(c). From a consideration of the survivor curves, additional evidence was obtained to support the contention that the concentration of undissociated hypochlorous acid was probably the determining factor in the rate of disinfection by hypochlorites.

Oxidation potentials of chloramine-T solutions were found to be unsatisfactory as measures of germicidal activity, since doubling the concentration reduced the killing time almost to one-half whereas the oxidation potential was not significantly altered.

Solutions of chloramine were strongly germicidal, and this germicidal power was less affected by pH changes than was the case with the hypochlorite and chloramine-T solutions.

Concerning the relative germicidal powers of the three types of chlorine compounds which were studied, in solutions practically free from organic matter, it was found that for a given concentration of available chlorine:

(a). Chloramine-T exhibited the weakest germicidal action.

(b). Monochloramine was more strongly germicidal than hypochlorites when the comparisons were made in highly alkaline solutions. In less alkaline solutions, at reactions below the range pH 9.5 to 10.0, hypochlorites were more germicidal than monochloramine or the resulting mixtures of monochloramine and dichloramine.

The nature of the survivor curves (log survivors against time) was not the same in disinfection with the various types of chlorine compounds studied. The survivor curves obtained in the experiments with monochloramine simulate a straight line and resemble those obtained with chloramine-T. The hypochlorite survivor curves show a great deviation from a straight line relationship, particularly at the higher alkalinities where a pronounced lag followed by a rapidly increasing death rate is noted. A fundamental difference in the mechanism of disinfection with the two general types of chlorine compounds is thereby indicated.

In disinfection with chloramine and chloramine-T hypochlorous acid does not appear to play the significant role which it exhibits with hypochlorites.

Disinfecting action for all of the compounds appears to be associated with the presence of a positive chlorine atom.

AMINO AND HYDROXY DERIVATIVES OF DIBENZOFURAN[1]

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Orientation studies have disclosed that all of the positions in the dibenzofuran nucleus, with the exception of the 1- and 9-positions, are vulnerable to direct nuclear substitution. An inspection of the complex morphine structure impresses one with the necessity of being able to substitute the (so-called critical) 1-, 4-, 6-, and 9-positions of dibenzufuran in order to synthetically approach the constitution of this important alkaloid.

The objectives of the orientation and metalation studies of this investigation were to render 4,6-disubstituted dibenzofurans more available, to prepare certain 1,4,6,9-tetra-substituted dibenzofurans, and to explore the feasibility of synthesizing 4,5-phenanthrylene oxide and 4,5-phenanthridine oxide derivatives through cyclization methods applied to the 1- and 9-positions of 4,6-disubstituted dibenzofuran derivatives, that is, 4,6-dimethoxydibenzofuran. Amino and hydroxy derivatives were chosen because of their inherent ability to activate the aromatic nucleus. The compounds were also to be submitted for pharmacological test, and a review of useful analgesics, coupled with the results of previous physiological tests on dibenzofurans, favored the conclusion that amino and hydroxy derivatives were most apt to manifest analgesic activity.

An attempt has been made to tabulate all amino and hydroxy derivatives of dibenzofuran which have appeared in the literateure prior to May, 1938. In addition, a number of compounds taken from unpublished work in this laboratory have been included along with the new compounds described in the thesis. All available references to each individual compound have been collected. Because of the inordinate task of compilation, the brazans, the dinaphthylene oxides, the morphine alkaloids, and many other derivatives of fused ring systems inhering a dibenzofuran nucleus have been omitted. *Chemical Abstracts* has been the criterion in deciding which compounds should be included.

The likelihood of linking together the 1- and 9-positions of dibenzofuran by means of a two-carbon bridge for the synthesis of 4,5-phenanthrylene oxides finds the support of analogy in the literature. Pertinent literature citations to analogous cyclizations have been presented.

The preparations of the following amino and hydroxy derivatives of dibenzofuran have been described: 4,6-dihydroxydibenzofuran (m.p. 200-202°), 3-hydrazinodibenzofuran (m.p. 174-175°), 1,2,3,4-tetrahydro-6-aminodibenzofuran hydrochloride (m.p. 227-228° decompn.), bi-(4-dibenzofuryl) (m.p. 191°), di-(methoxy-1-dibenzofuryl) ketone (m.p. 234°), bi-(4-methoxy-1-dibenzofuroyl) (m.p. 329°), bi-(4,6-dimethoxy-1-dibenzofuroyl) (m.p. above 300°), 1-chloroacetyl-4-methoxydibenzofuran (m.p. 165- 166°), 1-ethoxalyl-4-methoxydibenzofuran (m.p. 113°), 4-methoxy-1-dibenzofuryl-α-oxoacetic acid (m.p. 187°), 4-methoxy-diben-

[1] Original thesis submitted June, 1938. Doctoral thesis number 474.

zofuryl-α-oxoacetic acid semicarbazone (m.p. 211-212° decompn.), 3-hydroxy-4-methoxydibenzofuran (m.p. 109-110°), bi-(6-methoxy-4-dibenzofuryl) (m.p. 237-238°), bi-(6-hydroxy-4-dibenzofuryl) (m.p. 285-286°), 3,4-dimethoxydibenzofuran (m.p. 60-61°), 3,4-dihydroxydibenzofuran (m.p. 164-164.5°), 4,6-dimethoxydibenzofuran (m.p. 128-129°).

4,6-Dimethoxydibenzofuran picrate (m.p. 161-162°), 4-bromo-6-methoxydibenzofuran (m.p. 114°), 4-bromo-6-hydroxydibenzofuran (m.p. 138-139°), 4-amino-6-methoxydibenzofuran (m.p. 109°), 4-amino-6-hydroxydibenzofuran (m.p. 191.5-192.5°), 1-bromo-3,4-dimethoxydibenzofuran (m.p. 108°), 1-bromo-3-hydroxy-4-methoxydibenzofuran (m.p. 161-162°), 1-bromo-4,6-dimethoxydibenzofuran (m.p. 152°), 1,9-dibromo-4,6-dimethoxydibenzofuran (m.p. 167-168°), 1,9-dibromo-4,6-dihydroxydibenzofuran (m.p. 239-240°), 1,3 (?)-dibromo-4-hydroxy-6-methoxydibenzofuran (m.p. 177-178°), 1,3 (?)-dibromo-4,6-dimethoxydibenzofuran (m.p. 173.5-174°), 4,6-diaminobenzofuran (m.p. 152°), 4,6-diaminodibenzofuran picrate (m.p. 213° decompn.), 4,6-diacetaminodibenzofuran (m.p. 297-298°), 4,6-diacetoxydibenzofuran (m.p. 177°), 3,4- diacetoxydibenzofuran (m.p. 104-105°).

1-Acetyl-4,6-dimethoxydibenzofuran (m.p. 178-179.5°), 1-acetyl-3,4-dimethoxydibenzofuran (m.p. 90.5-91°), 1-acetyl-4,6-dimethoxydibenzofuran oxime (m.p. 203-204°), 1-acetyl-3,4-dimethoxydibenzofuran oxime (m.p. 156-157°), 1-acetamino-4,6-dimethoxydibenzofuran (m.p. 244-245°), 1-acetamino-3,4-dimethoxydibenzofuran (m.p. 196-196.5°), 1-benzeneazo-4-hydroxy-6-methoxydibenzofuran (m.p. 175°), 1-benzeneazo-4,6-dimethoxydibenzofuran (m.p. 170°), 1-amino-4,6-dimethoxydibenzofuran (m.p. 162-162.5°), 1-amino-3,4-dimethoxydibenzofuran (m.p. 162.5-163°), 4,6-dimethoxy-1-dibenzofurancarboxylic acid (m.p. 297- 298°), methyl 4,6-dimethoxy-1-dibenzofurancarboxylate (m.p. 163°), diazomethyl 4,6-dimethoxy-1-dibenzofuryl ketone (m.p. 151° decompn.), 4,6-dimethoxy-1-dibenzofurylacetamide (m.p. 210-211°), 4,6-dimethoxy-1-dibenzofurylacetic acid (m. p. 205-206°), 1,3,9-tribenzeneazo-4,6-dimethoxydibenzofuran (m.p. 191-193°) and di-(4,6-dimethoxy-1-dibenzofuryl) ketone (m.p. 254-255°).

Several attempts to prepare a phenanthrylene oxide derivative by intramolecular alkylation of 1-chloroacetyl-4-methoxydibenzofuran in intramolecular alkylation of 1-chloroacetyl-4-4methoxydibenzofuran in the presence of aluminum chloride emphasized the noteworthy inertness of the chlorine atom under the conditions of the Friedel-Crafts reaction. In every case either starting material was recovered or hopeless decomposition took place.

The isolation of bi-(4-methoxy-1-dibenzofuroyl) from the Friedel-Crafts reaction of 4-methoxydibenzofuran with oxalyl chloride suggested that in order to realize the formation of the desired *o*-quinone with oxalyl chloride, both the 1- and 9-positions of the dibenzofuran nucleus should be activated by the introduction of suitable substituents in the 4- and 6-positions. Consequently, 4,6-dimethoxydibenzofuran was prepared and subjected to the Friedel-Crafts reaction. Again intermolecular condensation proceeded with the formation of bi-(4,6-dimethoxy-1-dibenzofuroyl) in excellent yield. Likewise, failure attended three efforts to synthesize a 4,5-phenanthridine oxide derivative from 1-aceta-

mino-4,6-dimethoxydibenzofuran by application of the Bischler-Napieralski reaction.

The synthesis of 4,6-dimethoxy-1-dibenzofurylacetic acid has supplied a compound which holds unprecedented promise of yielding a 4,5-phenanthrylene oxide derivative. One unsuccessful preliminary attempt to achieve ring closure has been described.

Because of the powerful *ortho*-orienting influence exerted by ether groups in organoalkali metalations, the metalation of 4-methoxydibenzofuran with *n*-butyllithium followed by subsequent oxidation of the product produced the two isomers, 3-hydroxy-4-methoxydibenzofuran and 4-hydroxy-6-methoxydibenzofuran in almost equal quantity. The unexpected isolation of bi-(6-methoxy-4-dibenzofuryl) from the same reaction product led to the discovery that considerable bi-(4-dibenzofuryl) is formed during the preparation of 4-hydroxydibenzofuran. A mechanism for this definitely established coupling reaction has been proposed.

THE GROSS ANATOMY OF CORIZUS LATERALIS (SAY) (HEMIPTERA, CORIZIDAE)[1]

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Corizus lateralis (Say) is a small Hemipteron which occurs in many localities in the United States. It is found more easily on smart weed (*Polygonum pennsylvanicum* L.) than on any other plant. Because the insect is plentiful, readily kept in captivity, and because very little work has been done on the anatomy of the family Corizidae, the writer chose this species for a morphological subject.

Dissections were made after imbedding the insect just below the surface of beeswax in a Syracuse watch glass. This wax matrix held the small specimens firmly while they were being dissected. Boiling the insects for fifteen minutes hardened the internal organs to facilitate dissecting.

Micro-scalpels were improvised by breaking thin safety razor blades into small angular fragments, which were fastened to the tips of discarded dental scrapers by means of "Liquid Solder," a metallic cement. Microneedles were made the same way, using "minuten" insect pins instead of the pieces of razor blades.

The n-butyl alcohol method proved satisfactory in making serial sections. Morty-micra sections were of more help than thinner ones for observing internal organs.

It was found that the wing veins could be observed very easily if the wings were mounted on a layer of gylcerine jelly and covered with a cover glass so that the mounting medium was on only one surface of the wing.

An improvised microprojector was used in making most of the drawings. A compound microscope was placed horizontally, with a small focusing microscope lamp (six volts) as a source of concentrated brilliant illumination. A silvered prism at the ocular turned the rays down on the drawing paper. The size of the colored images could be changed by varying the projection distance.

Photomicrographs were made with the above described projection apparatus by using photographic bromide enlarging paper in lieu of drawing paper.

The anatomy of *Corizus lateralis* was found to be very complicated. The head is composed of inseparable sclerites. Two large compound eyes on each side of the head, two ocelli in the dorsal area between the eyes, and two six-jointed antennae constitute the more evident sensory structures of the head.

The beak is made up of a four-segmented labium which has a groove on its anterior surface to enclose the two mandibular and two maxillary bristles. These bristles interlock to form a food canal and a salivary canal running the length of the beak.

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The labial or salivary glands are located in the cephalic portion of the abdomen, with only two lobes resting in the thorax. Two salivary ducts connect the glands with the salivary syringe in the head.

There are no cervical sclerites in *Corizus lateralis*. The prothorax is very broad and large. The mesothorax and metathorax are combined into a pterothorax, bearing the four wings.

There is a pair of scent gland ostioles in the anterior pleural portions of the metathorax. The granular surrounding areas probably serve as evaporating plates.

The repugnatory or scent gland is located in the metathoracic sternum. Composed of two convoluted tubules, the gland rests between the sternal furca, with the reservoir almost covering both tubules. A valvular mechanism allows the secretion to be ejected from the reservoir.

Coxa, trochanter, femur, tibia, and tarsus compose the legs. The pretarsus is modified for locomotion by means of claws and pulvilli.

Basally, the fore wings are coriaceous with large veins, but the terminal portions are membranous. The hind wings are composed of three main regions—the remigium, vannus, and jugum. Vannal and jugal folds separate the wing areas. The fore and hind wings have a coupling device for uniting the two wings on each side into one functional unit.

Six segments of the abdomen are easily seen. The first is partially hidden by the metathorax. Dorsally, the fourth segment is concave at the base and at the apex, corresponding with the presence of a small dorsal scent gland. Segments seven and eight are usually retracted into the sixth and are modified for reproduction. The pleural regions form a prominent ridge or connexivum on each side of the abdomen, producing a trough-like tergum which is covered by the resting wings.

The alimentary canal is divided into the buccal cavity, crop, proventriculus, ventriculus, anterior intestine, posterior intestine, rectum, and anus. A stomodaeal valve is in the anterior part of the ventriculus, while a pyloric valve designates its caudal end.

Four malpighian tubules are connected with the anterior intestine. These tubules do not coalesce at their distal ends as they do in some Hemiptera. Rectal papillae are not present.

The heart is located in the dorsal section of the abdomen, with a slender aorta leading to the brain. Dorsal diaphragm muscles hold the heart in position. When viewed laterally, the ostia appear as tiny vertical elliptical perforations. Normally, the heart rate is eighty-five times per minute. Variations in temperature cause fluctuations in the number of pulsations. No reversal of flow was noticed. Numerous pericardial cells surround the heart. The aorta is located in the thorax and head, and is smaller than the heart, having no ostia or chambers.

The brain is divided into the protocerebrum, the deutocerebrum, and the tritocerebrum, which are not sharply constricted from each other.

Numerous nerves originate in the brain. A small frontal ganglion lies just in front. Short, thick, circumoesophageal connectives link the brain and the suboesophageal ganglion.

A single ganglion rests in the prothorax. The ganglia of the mesothorax and metathorax are combined to form a large pterothoracic ganglion. Because of a transverse constriction, this appears as two sep-

arate ganglia. Posteriorly, a large median nerve sends branches into the abdominal segments.

Tiny spindle-shaped ovarioles make up the ovaries. Anteriorly, the ovarioles taper into slender terminal jlaments which end in the thorax. The ovariole ducts or pedicels units to form two common lateral oviducts which fuse to produce the oviductus communis. An accessory gland is located on the floor of the sixth segment. Part of the spermathecal duct coils spirally around the spermatheca.

The testes are bright red and are composed of seven sperm tubes. They are divided into the germarium, zone of growth, maturation zone, and zone of transformation. Vasa deferentia connect the testes with the ejaculatory reservoir. A ductus ejaculatorium carries the spermatozoa from the reservoir to the penis.

EFFECT OF PLANT GROWTH-PROMOTING SUBSTANCES ON VITAMIN CONTENT AND REPRODUCTION OF LEMNA[1]

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Plant growth-promoting substances of various kinds are formed in the shoots and roots of germinating corn (auxins) and in beef liver (pantothenic acid). These, together with related synthetic organic compounds, were used in inorganic media to determine their influence on the rate of reproduction of Lemna major grown in sterile cultures under electric light.

Vitamin B_1 has been shown to be a growth-promoting substance for excised roots. The production of vitamin B_1 in Lemna major, grown under varied conditions, was determined by rat-feeding experiments.

INFLUENCE OF EXTRACT OF BEEF LIVER (PANTOTHENIC ACID) ON GROWTH OF LEMNA

Williams (8) designated as "pantothenic acid" a growth-promoting factor for yeast which he prepared in a crude concentrated form by fractional electrolysis of an 80 per cent methyl alcohol extract of plant and animal tissue. The acid was concentrated in the acidic electroylzed fractions and found to stimulate the growth of the yeast and also the growth of alfalfa seedlings (McBurney, 5). An 80 per cent methyl alcohol extract of beef liver, a material found by Williams (8) to contain pantothenic acid, was used as a source of this growth substance for experiments with Lemna. A water solution of the extract was concentrated by fractional electrolysis (Williams, 8).

Lemna, free from microorganisms, were grown in Clark's (3) sterile solution, with additions from the electrolyzed fractions of the liver extract, at a pH of 4.8. The plants were grown for four weeks under controlled environment (25° C. and 14½ hours exposure daily to mazda illumination of 150 foot-candles intensity), and were transferred to freshly prepared solutions at five day intervals (Clark, 2). The rate of reproduction of each culture was determined graphically by the method of Clark (3).

The rate of increase was stimulated by both the acidic and the alkaline fractions from the electrolyzed liver extract. Williams (8) did not report a marked stimulation of yeast from alkaline fractions but these gave a definite stimulation of the Lemna. McBurney (5) used only the acidic fractions for alfalfa seedlings and secured a stimulation of growth accompanied by a decrease in chlorophyll. The stimulation of Lemna was also accompanied by a loss of chlorophyll as well as a decrease in the size of fronds and inhibition of root growth.

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INFLUENCE OF AUXINS ON GROWTH OF LEMNA

The roots and shoots of germinated corn were used as a source of auxins, the plant growth-promoting substances discovered in the tips of oat coleoptiles by Went (7). Water solutions of a 95 per cent ethyl alcohol extract of the materials were concentrated by fractional electrolysis as before. Sterile Lemna were grown in inorganic media supplemented with the prepared extracts. The inorganic media and the growth conditions were identical with those outlined for the pantothenic acid experiments.

From the shoots of corn the auxins were not concentrated in any of the cells by the fractional electrolysis; from the corn roots there was some indication of concentration but no regularity. Possibly the growth substances from the two sources were not identical, but the effect on the Lemna was the same.

The prepared extracts increased the rate of reproduction of Lemna but in both cases inhibited the growth of roots—an inhibitory effect reported by Thimann (6) for oat seedlings in auxin solutions. The stimulation of Lemna by the auxins was accompanied by a decrease in both chlorophyll and size of fronds. These effects were similar to those of the beef liver extract.

INFLUENCE OF SYNTHETIC GROWTH-PROMOTING SUBSTANCES ON REPRODUCTION OF LEMNA

Beta-indolylacetic, phenylacetic and phenylpropionic acids, organic compounds known to stimulate the growth of green plants, were added to sterile inorganic media in concentrations varying from 0.0001 to 100 mg. per liter of media. Water solutions of the compounds were sterilized by filtration through Pasteur Chamberland filter candles and combined aseptically with the sterile inorganic media.

Beta-indolylacetic acid (identical with heteroauxin, one of the auxins produced in the coleoptile tips of Avena), in any of the concentrations used did not stimulate the rate of reproduction of the Lemna; 0.1 and 1.0 mg. per liter of media caused a marked inhibition of root growth and a decrease in both chlorophyll and size of fronds, and 10 mg. or more killed the plants.

The lower concentrations (0.0001 mg. up) of phenylacetic and phenylpropionic acids produced a very slight increase in the rate of reproduction of Lemna, while 0.1 and 1.0 mg. per liter of media decreased that function and inhibited root growth, but increased frond size. Ten mg. per liter of phenylacetic acid and amounts somewhat higher than 10 mg. for phenylpropionic acid, were toxic to the plants.

No permanent changes were effected in Lemna by concentrations of the synthetic compounds lower than the amounts which killed the plants. The treated Lemna, when transferred to inorganic media, returned to normal health and growth within three weeks.

The results obtained from the stimulation of Lemna, both by the growth-promoting extracts and by the pure synthetic growth-promoters, indicated that any marked effects produced were at the expense of some other part or function of the plant. There is thus some confirmation of Leonian's (4) view that the synthetic auxins may be frequently growth-inhibiting rather than growth-inducing.

SYNTHESIS OF VITAMIN B_1 BY LEMNA GROWN UNDER VARIED CONDITIONS

The presence of vitamin B_1 in green plants and its relation to plant growth-promoting substances (Bonner, 1) suggested that healthy and normal Lemna should synthesize the vitamin regardless of conditions of growth.

Lemna were grown under the environmental conditions described for "Pantothenic Acid," and also in a soil-water mixture in sunlight in the presence of microorganisms and organic matter. Excess plants from these cultures were air-dried and included in the diet of vitamin B_1-depleted rats as the only source of that vitamin. Under the growth conditions used, the results from two rat feeding experiments showed that vitamin B_1 was synthesized by Lemna and that a greater quantity was produced by sterile plants grown in inorganic media under electric light than by non-sterile plants grown in a soil-water mixture in sunlight.

SUMMARY

1. An extract of beef liver (pantothenic acid) and extracts of roots and shoots of germinated corn (auxins) increased the rate of reproduction of Lemna. The stimulation was accompanied by an inhibition of root growth and a decrease in both chlorophyll and size of the fronds.

2. Beta-indolylacetic acid (synthetic heteroauxin) in concentrations from 0.0001 to 100 mg. per liter of media did not stimulate the reproduction of the plants; 0.1 and 1.0 mg. inhibited root growth and decreased both chlorophyll and size of fronds, and 10 mg. or more killed the plants.

3. Phenylacetic and phenylpropionic acids in concentrations of 0.0001 to 0.01 mg. per liter of media gave a very slight stimulation to the rate of reproduction; 0.1 and 1.0 mg. per liter decreased that function, inhibited root growth and caused an increase in size of fronds. Ten mg. or more of phenylacetic acid and amounts somewhat higher than ten mg. of phenylpropionic acid were toxic.

4. Lemna, irrespective of the presence or absence of microorganisms and organic matter, were shown to synthesize vitamin B_1. A greater quantity was produced by sterile plants in inorganic media than by non-sterile Lemna in a soil-water mixture.

LITERATURE CITED

1. Bonner, J.
   1937. Role of vitamins in plant development. Bot. Rev. 3:616-640.

2. Clark, N. A.
   1932. Technique for the growth of Lemna under sterile conditions with controlled temperature and light. Iowa State Coll. Jour. Sci. 7:13-16.

3. Clark, N. A., and E. M. Roller
   1931. The stimulation of Lemna major by organic matter under sterile and non-sterile conditions. Soil Sci. 31:299-309.

4. Leonian L. H., and V. G. Lilly
   1937. Is heteroauxin a growth promoting substance? Amer. Jour. Bot. 24:135-139.

5. McBurney, C. H., W. B. Bollen and R. J. Williams
1936. Pantothenic acid and the nodule bacteria-legume symbiosis. Proc. Natl. Acad. Sci. 21:301-304.

6. Thimann, K. V.
1937. On the nature of inhibitions caused by auxin. Amer. Jour. Bot. 24:407-412.

7. Went, F. W.
1935. Auxin, the plant growth hormone. Bot. Rev. 1:162-182.

8. Williams, R. J., C. Lyman, G. Goodyear, J. Truesdail and D. Haladay
1933. "Pantothenic Acid." A growth determinant of unviersal biological occurrence. J. Amer. Chem. Soc. 55:2912-2927.

STRESSES IN MODERATELY THICK RECTANGULAR PLATES[1]

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In 1922 G. D. Birkhoff (1) suggested a method for solving plate problems which involves the representation of the displacements by power series. C. A. Garabedian (2) and H. W. Sibert (3) used this idea in developing methods for solving problems in moderately thick circular plates. Garabedian (4) has also published some results for uniformly loaded rectangular plates.

The author gives a solution for the displacements in an elastic isotropic moderately thick rectangular plate under the action of any given load which can be expressed as a polynomial in x,y continuous over the entire plate and with prescribed boundary conditions at the edges. The method, similar to that used by Sibert (3) for circular plates, is based on the assumption that the components of displacement can be developed in positive integral powers of z. In this type of problem, the displacements must satisfy (a) the stress equations of equilibrium throughout the plate, (b) the surface traction conditions on the upper and lower faces, and (c) the boundary conditions at the edges.

GENERAL THEORY

The displacements, u, v, w, are given by

$$(1) \qquad u = \sum_{n=0}^{\infty} U_n \frac{z^n}{n!}, \qquad v = \sum_{n=0}^{\infty} V_n \frac{z^n}{n!}, \qquad w = \sum_{n=0}^{\infty} W_n \frac{z^n}{n!},$$

where U_n, V_n, and W_n are continuous and continuously differentiable functions of x,y. When these displacements are substituted in the equations of equilibrium (A. E. H. Love (5), p. 134) recurrence relations are obtained by which U_n, V_n, and W_n are expressed in terms of U_0, V_0, W_0, U_1, V_1, and W_1.

A right-handed coordinate system with the faces of the plate $z = \pm h$, $x = o$, $x = a$, $y = o$, and $y = b$ is used. When the displacements are made to satisfy the surface traction conditions (Love (5), p. 77 and p. 101) two simultaneous systems of partial differential equations are obtained. One system involves only W_0, U_1, and V_1; the other, only U_0, V_0, and W_1.

For simplicity the problem is restricted to the case of a normal surface load only. The solution for the case of a shearing load is very similar to this case. By superposing these solutions, the results for more complicated problems can be obtained.

The general solutions of the two simultaneous systems of equations are obtained by an indirect process due to Sibert (3).

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Let $\Delta_2 = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2}$; $D = \frac{4\mu h^3}{3(1-\sigma)} = \frac{2Eh^3}{3(1-\sigma^2)}$; $-p_1$ and $-p_2$, where p_1 and p_2 are functions of x,y, equal the difference and sum, respectively, of the normal loads on the top and bottom of the plate. The general solutions are

$$\Delta_4 W_0 = \sum_{n=0}^{\infty} \frac{h^{2n}\Delta_{2n}p_1}{D}\left[(2-\sigma)d_n - b_n\right], \tag{2}$$

$$\Delta_4 U_1 = \sum_{n=0}^{\infty} \frac{h^{2n}\Delta_{2n}\left(\frac{\partial p_1}{\partial x}\right)}{D}\left[\sigma d_n + b_n\right], \tag{3}$$

$$\Delta_4 V_1 = \sum_{n=0}^{\infty} \frac{h^{2n}\Delta_{2n}\left(\frac{\partial p_1}{\partial y}\right)}{D}\left[\sigma d_n + b_n\right], \tag{4}$$

$$\Delta_2\left(\frac{\partial U_1}{\partial x} + \frac{\partial V_1}{\partial y}\right) = \sum_{n=0}^{\infty} \frac{h^{2n}\Delta_{2n}p_1}{D}\left[\sigma d_n + b_n\right], \tag{5}$$

$$\Delta_2 W_1 = -\sum_{n=0}^{\infty} \frac{h^{2n}\Delta_{2n+2}\,p_2}{4\mu}\left[(1-\sigma)c_n + \sigma a_n\right], \tag{6}$$

$$\Delta_4 U_0 = \sum_{n=0}^{\infty} \frac{h^{2n}\Delta_{2n+2}\left(\frac{\partial p_2}{\partial x}\right)}{4\mu}\left[\sigma c_n + (1-\sigma)a_n\right], \tag{7}$$

$$\Delta_4 V_0 = \sum_{n=0}^{\infty} \frac{h^{2n}\Delta_{2n+2}\left(\frac{\partial p_2}{\partial y}\right)}{4\mu}\left[\sigma c_n + (1-\sigma)a_n\right], \tag{8}$$

$$\Delta_2\left(\frac{\partial U_0}{\partial x} + \frac{\partial V_0}{\partial y}\right) = \sum_{n=0}^{\infty} \frac{h^{2n}\Delta_{2n+2}\,p_2}{4\mu}\left[\sigma c_n + (1-\sigma)a_n\right], \tag{9}$$

where $a_0 = 0$, $b_0 = 1$, $c_0 = 1$, $d_0 = 0$,

$$a_n = \sum_{i=0}^{n-1} (-1)^i \frac{(i+2)a_{n-1-i} - (i+1)c_{n-1-i}}{(2i+3)!} \qquad (n = 1, 2, 3, \ldots),$$

$$b_n = 6\sum_{i=0}^{n-1} (-1)^i \frac{(i+2)\left[(i+2)b_{n-1-i} - (i+1)(1-\sigma)d_{n-1-i}\right]}{(2i+5)!} \qquad (n = 1, 2, 3, \ldots),$$

$$c_n = \sum_{i=0}^{n-1} (-1)^i \frac{(i+1)a_{n-1-i} - ic_{n-1-i}}{(2i+2)!} \qquad (n = 1, 2, 3, \ldots),$$

$$d_n = \sum_{i=0}^{n-1} (-1)^i \frac{(i+1)b_{n-1-i} - i(1-\sigma)d_{n-1-i}}{(1-\sigma)(2i+2)!} \qquad (n = 1, 2, 3, \ldots).$$

The displacements u, v, w are readily obtained when the six functions U_0, V_0, W_0, U_1, V_1 and W_1 are known. Therefore, one can say that the differential equations (2) to (9) inclusive define the displacements. Furthermore, the displacements defined by these differential equations satisfy the equilibrium equations and the surface traction conditions for any normal load which can be expressed as a polynomial in x, y continuous over the entire plate. It remains to solve these differential equations subject to particular sets of edge conditions.

NORMAL LOAD, $p_1(x,y) = p_2(x,y) = P\left(\lambda + \frac{\alpha x}{a} + \frac{\beta y}{b}\right)$

The displacements are found for a normal load $P\left(\lambda + \frac{\alpha x}{a} + \frac{\beta y}{b}\right)$ on the top surface of the plate where a and b are the horizontal dimensions of the plate, λ, α, and β are arbitrary constants, and P is a uniform load per unit of area. In each case the edges $x = o,a$ are pinned. The three cases where the edges $y = o,b$ are pinned, clamped, and free are solved. In each case the moderately thick plate solution consists of the corresponding thin plate solution plus corrections. Each moderately thick plate solution reduces to that given by Garabedian (4) for the case $\lambda = 1$ and $\alpha = \beta = o$.

LITERATURE CITED

1. Birkhoff, G. D.
   1922. Circular plates of variable thickness. Phil. Mag. Series 6, 43:953-962.

2. Garabedian, C. A.
   1923. Circular plates of constant or variable thickness. Trans. Am. Math. Soc., 25:343-398.

3. Sibert, H. W.
   1931. Moderately thick circular plates with plane faces. Trans. Am. Math. Soc., 33:329-369.

4. Garabedian, C. A.
   Compt. rend. Acad. sci. Paris, 178(1924), 180(1925), 181(1925), 186(1928), 195(1932.)

5. Love, A. E. H.
   1927. A treatise on the mathematical theory of elasticity. 4th ed. University Press, Cambridge.

SULFUR ANALOGS OF FURAN TYPES[1]

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The chemistry of furan analogs has been extended in an investigation of the sulfur analog, dibenzothiophene. A review of the natural occurrence and methods of preparation of dibenzothiophene, and of several general methods for the ring closure synthesis of dibenzothiophene derivatives has been made.

The most extensive study of the orientation in dibenzothiophene is that of Courtot and co-workers. They have shown that halogenation, nitration, and sulfonation affect the 2- and 2,8-positions (*para* to the sulfur linkage). In extending this study it has now been found that acetylation by the Friedel-Crafts reaction, using acetyl chloride and anhydrous aluminum chloride in carbon disulfide gives 2-acetyldibenzothiophene, m.p. 111°. The structure of this ketone was established by oxidation with iodine and sodium hydroxide to dibenzothiophene-2-carboxylic acid, first prepared by Courtot, Nicolas and Liang[2]. Methyl dibenzothiophene-2-carboxylate, m.p. 74-75°.

Dibenzothiophene has been found to undergo metalation with various organolithium compounds to give, after carbonation with solid carbon dioxide, dibenzothiophene-4-carboxylic acid, m.p. 252-253°; methyl ester, m. p. 95°. The structure of this acid was established by treating 4-dibenzothienyllithium with dimethyl sulfate to give 4-methyldibenzothiophene, m.p. 66.5°, and comparing it with an authentic specimen prepared by the reaction of 3-methyl-2,2′-dihydroxybiphenyl and phosphorus pentasulfide. The yields of dibenzothiophene-4-carboxylic acid from *n*-butyl-, phenyl-, α-naphthyl-, and *p*-anisyllithium were found to be approximately 65, 12, 7.6 and 0 per cent, respectively. An anomalous behavior of *p*-anisyllithium is noted which may account for the absence of any metalation.

Phenylcalcium iodide metalates dibenzofuran in the same position as do the organolithium compounds. However, treatment of dibenzothiophene with phenylcalcium iodide, followed by carbonation, gave a monobasic acid different from the 2- and 4-acids; m.p. 300-305° (dec.); methyl ester, m.p. 129-130°. Decarboxylation of the acid gave dibenzothiophene. This acid is probably dibenzothiophene-3-carboxylic acid.

Mercuration of dibenzothiophene by fusion with mercuric acetate gives what appears to be a monoacetoxymercuri derivative [m.p. 215° (dec.)] which is contaminated with a small amount of polymercurated material. The position of mercuration is unestablished.

Treatment of 4-dibenzothienyllithium with oxygen gives 4-hydroxydibenzothiophene, m.p. 167°. This phenol gives a green color with ferric chloride and, upon nitration with concentrated nitric acid in warm acetic acid solution, a dinitro derivatve, dark red crystals, m.p. 204° (dec.).

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[1] Original thesis submitted March, 1938. Doctoral thesis number 459

[2] Courtot, Nicolas and Liang, *Compt. rend.*, **186**, 1624 (1928).

Methylation of 4-hydroxydibenzothiophene gives 4-methoxydibenzothiophene, m.p. 123°.

When 4-hydroxydibenzothiophene is treated with sodium bisulfite and concentrated aqueous ammonia, 4-aminodibenzothiophene is obtained, m.p. 110°; acetyl derivative, m.p. 198°. A better synthesis of the 4-aminodibenzothiophene consists of treating crude 4-bromodibenzothiophene with concentrated aqueous ammonia and cuprous bromide in a bomb. The 4-bromodibenzothiophene is obtained by the reaction of bromine with 4-dibenzothienyllithium and need not be separated from the accompanying dibenzothiophene. Bromination of 4-acetaminodibenzothiophene gives a monobromo derivative, m.p. 254°, which is probably 1-bromo-4-acetaminodibenzothiophene.

With the original intention of preparing alkylated imidazoles for physiological tests, the nitration of 2-acetaminodibenzothiophene was studied. 2-Acetaminodibenzothiophene was first reported[3] as melting at 168° but was found to melt at 178°. Because of the difficulty of obtaining 2-nitrodibenzothiophene in good yield by direct nitration, for conversion into the desired amine, two new routes to the acetamino derivative were developed. The first involves the bromination of dibenzothiophene, amination of the bromo derivative with ammonia, and acetylation of the resulting amine. The other method consists of effecting the Beckmann rearrangement of the oxime (m.p. 160-161°) of 2-acetyldibenzothiophene. By either route, the overall yield of acetamino compound, based on the dibenzothiophene, is at least 38 per cent. Nitration of 2-acetaminodibenzothiophene with fuming nitric acid in acetic anhydride gives a nitro-acetamino compound, m.p. 209°, in 67 per cent yield, and a small amount of a nitro-aminodibenzothiophene is also formed, m.p. 248-250° (dec.). An attempt to hydrolyze nitro-2-acetaminodibenzothiophene with alcoholic hydrochloric acid gave an as yet unidentified nitrogen-free material, m.p. 88°.

Dibenzothiophene is best prepared by heating biphenyl with sulfur in the presence of aluminum chloride. The possibility of obtaining substituted dibenzothiophenes by starting with substituted biphenyls was investigated but the polymerizing effect of the cataylst under conditions necessary to cause an evolution of hydrogen sulfide seems quite marked, and from *p*-bromobiphenyl the only product which could be isolated was dibenzothiophene, in 15 per cent yield. The use of a milder condensing agent is apparently not practicable but there are some indications which point to the success of the method when chlorobiphenyls and a lower temperature are employed.

Two polycyclic, condensed-ring compounds containing the dibenzothiophene system have been synthesized for physiological tests. The first, 1-keto-1,2,3,4-tetrahydrothiobrazan (β- or γ-) was prepared as follows. Succinic anhydride was condensed with dibenzothiophene in the presence of anhydrous aluminum chloride to give β-2-dibenzothenoylpropionic acid, m.p. 160.5-161°. This was reduced with zinc and hydrochloric acid to give γ-2-dibenzothienylbutyric acid, m.p. 131°. Cyclodehydration of the latter with sulfuric acid gave the 1-keto-1,2,3,4-tetrahydrothiobrazan, m.p. 178°.

The second condensed-ring compound was prepared in a similar manner from dibenzothiophene and phthalic anhydride. The resulting *o*-2-

[3] Courtot and Pomonis, *Compt. rend.*, 182, 931 (1926).

dibenzothenoylbenzoic acid (unstable hydrate, m.p. 120-125°; ethyl ester, m.p. 105-106°) was cyclized by fusion with a mixture of aluminum chloride and sodium chloride to give yellow crystals of a thionaphthenoanthraquinone, m.p. 285-286°.

When dibenzothiophene is treated wth sodium in liquid ammonia a dihydro derivative is obtained, m.p. 76°; picrate, red needles, m.p. 105°. By analogy with naphthalene, phenanthrene, and dibenzofuran, this is probably 1,4-dihydrodibenzothiophene. When treated with bromine in chloroform solution at 0°, the dihydrodibenzothiophene absorbs a mole of bromine without evolution of hydrogen bromide. The resulting oil loses hydrogen bromide when warmed and gives an almost quantitative yield of dibenzothiophene. This behavior of the bromine addition product is analogous to that of the bromide of 1,4-dihydronaphthalene[4].

When 1,4-dihydrodibenzothiophene is treated with phenyllithium under conditions identical with those employed in metalations, dibenzothiophene is obtained in almost quantitative yield, along with an appreciable amount of benzene and a white solid which behaves like lithium hydride. It was subsequently shown that phenyllithium is cleaved by hydrogen in the absence of a catalyst to give benzene and lithium hydride. The readily obtainable phenylisopropylpotassium exerts a similar dehydrogenating effect on the dihydrodibenzothiophene.

Earlier work on the reduction of organometallic compounds has been done in this country by Adkins[5] In 1932 he established a series of organometallic compounds in the order of their decreasing ease of reduction as follows, R_2Mg, R_2Zn, R_4Pb. The compound RLi (phenyllithium) was found to apparently undergo reduction with greater ease than reported for the magnesium compound, thus bringing it into this series before the others. The series, it will be noticed, is then in the order of decreasing reactivity as established by other methods.

[4] Bamberger and Lodter, *Ber.*, 20, 1706 (1887).

[5] Zartmann and Adkins, *J. Am. Chem. Soc.*, 54, 3398 (1932).

TOXICITY OF METHYL FORMATE, ETHYLENE OXIDE AND METHYL BROMIDE, IN ADMIXTURE WITH CARBON DIOXIDE, TO THE RUST-RED FLOUR BEETLE[1]

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Received June 25, 1938

The literature relating to the use of CO_2 in admixture with fumigants shows not only that moderate concentrations of this gas may have a marked effect on the respiratory system of insects, but also that the quantity which is required to give the maximum insecticidal effect will vary with the kind of fumigant with which it is combined. The writer gives data on the proper proportions in which the CO_2 should be combined with methyl formate, ethylene oxide and methyl bromide, and shows that the stimulative effect produced by the CO_2 may be partly or wholly nullified if the percentage in the gaseous mixture is increased beyond the amount which is found to give the maximum increase in toxicity.

An analysis of the proprietary fumigant mixtures containing CO_2 in admixture with methyl formate, ethylene oxide and methyl bromide indicates that the percentage of CO_2 incorporated into these mixtures is based upon the quantity which is required to remove the fire-hazard of the fumigant, rather than upon the concentration which would affect the maximum increase in the toxicity of the fumigant. A better approach to the problem of determining the most effective fumigant-CO_2 mixture would be to ascertain the actual toxicological effects attending the exposure of insects to these fumigants when they are admixed with CO_2 in various proportions. The writer has attempted to do this in the present investigation.

The apparatus employed in the investigation was developed by the writer and has the advantage of permitting the vapors of volatile fumigants, instead of the liquid substance, to be introduced into partially evacuated 5-liter balloon flasks. The amounts are measured by means of a mercury manometer and the concentrations are expressed as milligrams per liter or per cent by volume.

Tests were first conducted to determine the concentrations of methyl formate, ethylene oxide and methyl bromide which are required to kill 100 per cent of the adults of the rust-red flour beetle *(Tribolium castaneum)* in 5 hours at a temperature of 27° C. The results are as follows: (1) methyl formate, 25.0 mg. per liter, (2) ethylene oxide, 17.5 mg. per liter and (3) methyl bromide, 8.75 mg. per liter.

The addition of CO_2 has a marked effect on the toxicity of methyl formate to the adults of the above insect. When 1 per cent of CO_2 is added to atmospheres containing 25.0 mg. of methyl formate per liter, the period of exposure required to kill all of the beetles is decreased to 3.5 hours, as compared with 5 hours when no CO_2 is present in the gaseous mixture. The addition of 5, 10, 20 and 40 per cent of CO_2 reduces the time

[1] Original thesis submitted December, 1937. Doctoral thesis number 454.

required to kill all of the insects to 2.5,2, 1.5 and .75 hours, respectively. Concentrations of CO_2 in excess of 40 per cent apparently impart no additional increase in toxicity to methyl formate.

The results obtained with ethylene oxide (17.5 mg. per liter) indicate that the addition of 1 per cent of CO_2 does not increase the toxicity of this chemical to *T. castaneum.* When 5 per cent of CO_2 is added to ethylene oxide, however, the length of time necessary to affect a 100 per cent kill of the beetles is reduced to 3 hours, as compared with 5 hours when no CO_2 is present. A further reduction in time to 1.5 hours is observed when the concentration of CO_2 is increased to 10 per cent. All of the insects are killed in three-fourths of an hour when CO_2 is admixed at the rate of 20, 40, 60, 80 and 99.8 per cent. The addition of 60 per cent of CO_2 apparently produces the maximum insecticidal effect with ethylene oxide. Mixtures containing 80 and 99.8 per cent of CO_2 appear to be somewhat less toxic than those which have 60 per cent incorporated into the gaseous mixture.

The results also show that the addition of CO_2 does not increase the toxicity of methyl bromide to the same extent as observed with methyl formate and ethylene oxide. Atmospheres of methyl bromide (8.75 mg. per liter) containing 1 per cent and 99.8 per cent of CO_2 have about the same degree of toxicity to *T. castaneum* and are only slightly more toxic than methyl bromide without CO_2. Both mixtures require 4.5 hours to kill all of the insects, as compared with an exposure period of 5 hours without the CO_2. Likewise, methyl bromide plus 80 per cent of CO_2 is apparently no more toxic than mixtures having 5 per cent of CO_2, as an exposure of 4 hours is required to kill 100 per cent of the beetles with each concentration. Ten to 40 per cent of CO_2 produce the maximum stimulative effect when used in admixture with 8.75 mg. of methyl bromide per liter, and the results indicate that mixtures containing 20 per cent are the most effective, especially in the lower range of mortalities.

The data presented in the thesis indicate, therefore, that the maximum insecticidal effect of methyl formate, ethylene oxide and methyl bromide is obtained with concentrations of 40, 60 and 20 per cent of CO_2, respectively, and that the addition of CO_2 in excess of these percentages may either impart no further increase in toxicity to the mixture, or may actually decrease the effectiveness of the gaseous mixture.

THE CRAWFISHES OF MISSISSIPPI, WITH SPECIAL REFERENCE TO THE BIOLOGY AND CONTROL OF DESTRUCTIVE SPECIES[1]

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Received June 25, 1938

This study of Mississippi crawfishes[2] resulted when a group of planters in the Northeast Prairie soil belt of Mississippi in 1932 requested the Mississippi Agricultural Experiment Station to develop control measures for a species which was very destructive to farm crops in that section. While conducting that investigation the writer became interested in learning how many species occurred in Mississippi. Collections were made in many parts of the state. The collection of the Zoology and Entomology Department of Mississippi State College, largely unidentified until the inception of this study, has been of much assistance. Comparisons of specimens with types in the Harvard Museum of Comparative Zoology and in the National Museum aided greatly with the identifications.

HISTORICAL

The earliest of any taxonomic work on crawfishes from Mississippi was by Charles Girard, who named the first species in 1852. In 1870 Hagen listed three species from the state in his "Monograph of the Astacidae." Faxon reported seven in 1885 and ten in 1914. The catalog of the National Museum now lists twelve authentic species. This study has increased the total number of known Mississippi species to 22, of which eight are new species and subspecies. Types are being retained at present in the collection of the Zoology and Entomology Department of Mississippi State College but will ultimately be deposited in the National Museum and the Harvard Museum of Comparative Zoology.

ECONOMIC IMPORTANCE

The only crawfishes in the United States greatly destructive to farm crops are *Cambarus hagenianus* Faxon and its subspecies, which apparently occur only in Mississippi and Alabama. Other species may drain ponds as a result of their burrows in dams, or may cause rivers at flood stage to break through levees weakened by their tunnels.

Of minor importance for human food, crawfish are very valuable in the diet of many animals such as raccoons, minks, opossums, bears and alligators. Wading birds, as well as some ducks, consume large numbers. Trout, bass, crappie, perch and other fish make them a staple item in their diets.

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[1] Original thesis submitted July, 1937. Doctoral thesis number 439.

[2] The term "crawfish" is used in this paper since it is the common name for these animals throughout the South and in other rural sections of the United States. It was early given approval in a scientific publication on Crustacea by Thomas Say in 1817.

GENERAL ANATOMY AND BIOLOGY

These subjects are treated in so many zoological texts it is unnecessary to discuss them here.[3]

CLASSIFICATION

Subgenera of the Genus *Cambarus* Erichson

All crawfishes in the United States east of the Rocky Mountains belong to the genus *Cambarus* Erichson. Mississippi apparently has representatives of all the subgenera known in the United States at this time. The following are recorded with the Mississippi species of each.[4]

Subgenus *Girardiella*, new subgenus (Faxon's Group II)

1. *Cambarus hagenianus* Faxon
2. *Cambarus hagenianus evansi*, new subspecies
3. *Cambarus hagenianus carri*, new subspecies
4. *Cambarus hagenianus forestae*, new subspecies

Subgenus *Ortmannicus* Fowler (Faxon's Group III)

5. *Cambarus blandingii acutus* Girard
6. *Cambarus hayi* Faxon
7. *Cambarus clarkii* Girard
8. *Cambarus cookae*, new species
9. *Cambarus evictus*, new species
10. *Cambarus spiculifer* LeConte
11. *Cambarus versutus* Hagen

Subgenus *Paracambarus* Ortmann (Faxon's Group IV)

12. *Cambarus harnedi*, new species

Subgenus *Cambarellus* Ortmann (Faxon's Group V)

13. *Cambarus shufeldtii* Faxon

Subgenus *Faxonius* Ortmann (Faxon's Group VI)

14. *Cambarus lancifer* Hagen
15. *Cambarus mississippiensis* Faxon
16. *Cambarus creaseri*, new species

Subgenus *Cambarus* Fowler (Faxon's Group VII)

17. *Cambarus diogenes* Girard
18. *Cambarus diogenes ludovicianus* Faxon
19. *Cambarus latimanus* LeConte
20. *Cambarus argillicola* Faxon
21. *Cambarus lobdelli*, new species

Subgenus *Faxonella* Creaser

22. *Cambarus clypeatus* Hay

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[3] The thesis contains a chapter on these subjects with an illustration of all the parts of a crawfish useful in identification.

[4] A key to the crawfishes of Mississippi, descriptions of new species, and distinguishing characters of others in the state, are included in the thesis.

THE BIOLOGY AND CONTROL OF CAMBARUS HAGENIANUS FAXON AND ITS SUBSPECIES

BIOLOGY

These crawfishes inhabit the lime soils of Mississippi and Alabama. Each subspecies is separated from the others but their habits are very similar. To secure information on their habits, flashlight observations on many rainy nights and the excavation of many hurrows were supplemented by laboratory studies of individuals in glass cages of soil standing in water. They live in deep burrows and never visit ponds or streams as do most other species. Their diet is chiefly vegetarian and on rainy spring nights they often destroy entire fields of young corn and cotton. They can live long periods without food, eight or nine months in some cases. They survive temperatures of 38° F. and probably lower but die quickly at 100-102° F. Only a small percentage of the females produce young each year. Oviposition occurs in the fall, the young usually hatching from October to December and remaining in the burrow with the mother until the winter or spring of the second year, or 12 to 18 months. The average number of young is about five or six, the maximum found in any nest being 20. Apparently they become sexually functional in about three years and attain full size when six or seven years old.

CONTROL

The best control is the application of poison to the burrows when the water level is only a few inches from the surface of the ground, or from January to May in most years. Pyrethrum, commercial creosote stock dips, coal tar creosote, orthodichlorobenzene, sodium cyanide, turpentine, ortho cresole, cresylic acid, miscible pine oil, nicotine, carbon bisulphide, phenothiazine and calcium cyanamid were toxic in laboratory tests in the order named. Ineffective materials included the arsenicals, rotenone, picric acid, pyridine, resorcinol, thallium sulphate and several others. Pyrethrum was ineffective at the temperatures in the burrows.

The creosote stock dips are recommended on account of their low cost and ease of application. With infestations of 10,000 burrows per acre the cost of material is about 50 to 60 cents per acre. Where the stock dips are not available at low cost, home-made emulsions of turpentine and creosote are advised.

Applications on small areas may be made with compressed air sprayers. On large plantations a double-acting force pump, mounted on a wagon and supplying several leads of hose, is very satisfactory. Splendid results have been secured on large-scale tests covering several hundred acres.

SOME FACTORS AFFECTING THE RAMAN FREQUENCIES OF THE CARBON-MERCURY BOND[1]

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Received June 25, 1938

Theoretical considerations indicate that Raman spectra studies should show how the carbon-mercury bond varies as the radical attached to the mercury is changed.

Hixon and Johns[2] found that the polar properties of a series of compounds such as RNH_2 or RCOOH which contain one polar group could be expressed as a function of the electron sharing ability of the radical R. Later studies on a number of equilibria supported the original hypothesis and confirmed the order in which the radicals were arranged. Studies of refractivity and thermal stability of compounds of the type R_2Hg and RHgCN by Carr[3] have added some purely physical measurements to the physico-chemical studies.

All these experiments measured the electron sharing ability indirectly. The present investigation was undertaken as a direct study of the bond between the radical and another group or atom. A series of R_2Hg compounds was chosen for the study because refractivity and thermal stabiltiy data were available for a number of these compounds.

The apparatus consisted of a high speed spectograph and an auxiliary excitation unit for producing the Raman spectra. The excitation unit was in the form of an elliptical cylinder and was made from polished sheet aluminum. The tube holding the liquid under investigation was placed in a vertical positon inside a condenser at one focus of the ellipse. The direct current mercury arc used as a light source was placed at the other focus of the ellipse. A total reflecting prism located directly below the Raman tube served to send light through a condensing lens into the spectograph. A slit in the elliptical reflector midway between the arc and the Raman tube allowed a glass light filter to be inserted. Solutions containing dissolved salts were circulated through the condenser to act as additional light filters and to keep the solution or liquid in the Raman tube at the proper temperature.

The following compounds' spectra were photographed: Diethylmercury, diethylmercury in carbon tetrachloride, diethylmercury in acetone, di-n-propylmercury, di-n-propylmercury in carbon tetrachloride, dibenzylmercury in carbon tetrachloride, dibenzylmercury in acetone, diphenylmercury in carbon tetrachloride, mercuric cyanide in acetone and mercuric chloride in acetone. Attempts were made to obtain the spectra of di-p-tolylmercury and di-alpha-naphthylmercury. Due to the very low solubility of these compounds no results were obtained for them.

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[1] Original thesis submitted June, 1938. Doctoral thesis number 476.

[2] Hixon and Johns, *J. Am. Chem. Soc.* **49**, 1786 (1925).

[3] Carr, Unpublished work, this laboratory. (1934.)

The frequencies observed for the compounds investigated are listed in table 1.

TABLE 1. *Frequencies of a series of organomercury compounds,* R_2Hg

Propyl	Ethyl	Benzyl	Phenyl	Chloride	Cyanide
171 (0)	140 (1)	175 (m)	152 (5)	321 (5)	276 (w)
276 (3)	212 (2)	260	340		
381 (3)	264 (3)	325	382		
501 (10)	329 (0)	560 (5)	555 (s)		
585 (4)	486 (8)	638	580		
791 (0)	562 (0)	690	619		
863 (1)	633 (0)	810	652 (s)		
1016 (2)	958 (1)	996 (s)	700		
1084 (2)	1008 (3)	1070	997 (s)		
1158 (10)	1055 (2)	1153	1060		
1267 (00)	1178 (6)	1205	1185		
1326 (00)	1370 (1)		1262		
1446 (2)	1421 (3)		1370		
	1455 (3)				

No separation of the frequencies listed in table 1 has been made on the basis of the solvent used. Variations due to the solvent were less than the experimental error in measuring the frequencies.

The molecules were considered as linear triatomic systems for the purpose of calculating force constants for the carbon-mercury bond. Table 2 shows the frequencies selected as the fundamentals and the force constants for the carbon-mercury bond. These constants are considered as representing the restoring forces which act on the group which vibrates.

TABLE 2. *Fundamental frequencies and force constants for* R_2Hg *compounds*

Radical	ν_1	ν_2	ν_3	k_1	k_α
Propyl	501	171	585	5.4	0.27
Ethyl	486	140	562	4.7	0.13
Methyl[4]	515	156	565	3.2	0.09
Benzyl	560	175	638	5.5	0.63
Phenyl	555	152	652	6.9	0.38
Chloride	321			2.2	
Cyanide	276			1.2	

In table 2, k_1 represents the stretching constant and k_α represents the bending constant. These and other constants calculated indicate that the radicals vibrate almost as units in the aliphatic compounds. The radicals do not vibrate as units in the aromtic compounds.

The values obtained for the bending constants indicate that the aliphatic compounds are much more flexible than the aromatic compounds.

[4] Thompson and Linnett, *Proc. Roy. Soc. (London)* **A 160**, 139 (1937).

The force constants and other information obtained from the Raman spectra were compared with the information given by thermal stability and refraction studies on the R_2Hg compounds.

If the radicals are placed in the order indicated by the dissociation constants of the amines the force constant curve shows a maximum. This maximum is at the same point as the minimum thermal stability and maximum refractivity of mercury in the same compounds.

CHARACTERIZATION OF CERTAIN PRODUCTS OF STARCH-ENZYME DIGESTION[1]

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Received June 25, 1938

Three fairly definite materials appear to be the result of beta amylase action on starches. They are: (1) The material which flocculates during the digestion, (2) 60-67 per cent maltose, (3) "residual dextrins" or "alpha amylodextrin." The purpose of this investigation was to prepare and study the flocculent material and residual material from beta amylase action on a series of starches.

The course of enzyme action was studied in order to determine the length of time to allow the enzyme to act and to determine any differences in beta amylase action on starches of different origin. The effects of the enzyme on viscosity, "residual starch"[2], and reducing action of the substrates were measured. However, the results of these determinations on the same digestion could not be correlated.

In the digestion of two per cent soluble potato starch with oat enzyme the viscosity had reached a minimum after 30 minutes of digestion, but the amount of material precipitated by 55 per cent alcohol was still decreasing after one hour. These two methods measure different effects of amylase action. Neither method is strictly a measure of the decrease in molecular size of the substrate as digestion proceeds. The reducing action against alkaline copper was still increasing after 50 minutes digestion. Similar experiments on the digestion of natural corn and potato starches with wheat amylase gave no correlation of the different methods. The results indicated that determinations of viscosity, "residual starch," and reducing action by Fehling's gravimetric method were not applicable to following the hydrolysis of natural starches.

Two other methods of following the reducing action during enzyme digestion were worked out. The modified Hagedorn and Jensen[3] sugar method gave satisfactory results during digestion of starches that had been boiled. However, when the substrates had been heated below the boiling point the results with this method were in error due to unswollen starch particles which held the iodine. A potentiometric sugar method was devised to remove this difficulty. The method of Hassid[4] was modified to a macro determination and the titration of ferrocyanide with ceric sulfate was followed potentiometrically.

A comparison of the rates of hydrolysis of natural and alcoholic HCl treated corn and potato starches was made. The substrates were prepared

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[1] Original thesis submitted June, 1938. Doctoral thesis number 472.

[2] Caldwell and Hildebrand, *J. Biol. Chem.* **111**, 411 (1935).

[3] Hagedorn and Jensen, *Biochem. Z.* **137**, 92 (1923).

[4] Hassid, *Ind. Eng. Chem. (Anal. Ed.)* **9**, 228 (1937).

by boiling, and the beta amylase from soybeans was used[5]. Changes in viscosity and reducing action as measured by the Hagedorn and Jensen method were followed. The decrease in viscosity was very markedly affected by the alcoholic HCl treatment of the starch. Liquefaction of the corn starches proceeded more rapidly than that of the potato starch, either treated or natural. The treatment of the starches had very little effect on the rate of sugar formation by beta amylase. Sugar production proceeded more rapidly during the digestion of corn starch than either natural or treated potato starch. However, after 24 hours the percentage reducing value as maltose was 66-68 per cent in the digestions of the unmodified starches. The value for the digestions of treated corn and potato starches was 61 per cent. It is possible that the treatment of the starches had washed out some more soluble portion of the starch which was digestible by beta amylase.

A study of the effect of temperature of preparing the substrate on the rate of beta amylase action on potato, corn, rice, wheat and tapioca starches was made. Substrates were prepared from these starches by heating 30 minutes at 60°, 70°, 80°, 90°, 100°, and 120° C. The rates of hydrolysis of the starch substrates by soybean amylase were measured by the potentiometric determination of the sugars formed. In every case except rice starch an optimum temperature of preparing the starch for soybean amylase action was indicated—70° for potato, 80° for tapioca, and 90° for corn and wheat starches. Temperatures of 80° or above gave the maximum rate of soybean enzyme hydrolysis. The optimum gelatinization temperatures were much lower for the root starches than for the cereal starches. However, the rates of soybean amylase action on the different substrates prepared at their optimum temperatures were all about the same. The final reducing values were all 60-70 per cent of the original starch. This series contained starches of very high and very low phosphorus and fatty acid content. If these groups are important in enzyme action, some differences should have been noted.

The material which flocculates during enzyme digestion of starches (precipitate A) and the residual material from beta amylase action which is precipitated by 60 per cent alcohol (precipitate B) were prepared from the same series of starches. The precipitates were repeatedly suspended in water and electrodialyzed in order to remove maltose and ions. The materials were finally dried by grinding under absolute alcohol and ether. When the ether was removed the materials were ground to white powders.

Precipitate A from the cereal starches was formed in larger amounts and settled out of the digestions. Precipitate A from the root starches was transparent when collected in the bowl of the supercentrifuge. Precipitate B from the root starches was a transparent sticky mass before dehydration. Precipitate B from the cereal starches was formed as a curdy white precipitate. One per cent suspensions of the dried powders showed differences also. The suspensions of precipitates A and B from cereal starches were turbid but not viscous. Suspensions of precipitate A from potato and tapioca starches were clear and quite viscous. Precipitate B from the root starches formed clear limpid suspensions.

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[5] The characterization of soybean amylase is unpublished work by Mr. J. M. Newton.

These preparations were characterized as to further hydrolysis by fresh beta amylase, reducing action against copper and ferrocyanide, phosphorus and fat content, and recovery in the starch determination of Denny[6]. These data support in the following ways the hypothesis that precipitate A is a portion of precipitate B which for some reason is thrown out of suspension.

1. Precipitates A and B from any one kind of starch are hydrolyzed by beta amylase to about the same degree. Here again the difference between cereal and root starches is noticeable. These materials from root starches are hydrolyzed to a much less degree than precipitates A and B from cereal starches.

2. The reducing values of precipitates A and B are about the same. This is confirmed by both potentiometric determinations and R_{Cu} values.

3. Precipitates A and B from any one kind of starch behave similarly in the starch determination of Denny. Precipitates A and B from root starches do not react at all in this determination.

4. The data on fat and phosphorus content of precipitates A and B indicate that the decreased solubility of precipitate A may be due to either fat or phosphorus. The presence of high content of fatty acids should make the material less soluble, while the phosphorus should make the materials more soluble.

[6] Denny, *J. Assoc. Official Agr. Chem.* **6**, 175 (1922).

FACTORS AFFECTING THE OCCURRENCE OF AZOTOBACTER IN IOWA SOILS[1]

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A previous investigation of the occurrence and distribution of Azotobacter in Iowa soils showed that many of the more important soil types of the state from the standpoint of total acreage were practically devoid of the bacteria. Barely a third of the 287 samples collected contained the bacteria. Correlation studies of the chemical composition of the samples and the presence of the Azotobacter indicated that the high acidity of the majority of these soils was the most important factor limiting the presence of the organisms and that the available phosphorus content was also of importance in many cases. Other factors being favorable, the results indicated further that the amount of growth which the Azotobacter would make in the soil depended largely upon the organic matter content and upon the pH.

The present investigation was planned to study further and in more detail the results of the correlation studies. Carefully controlled experiments were carried out in which the effect of various soil treatments upon the growth of Azotobacter while in the soil was determined.

The first studies were made of the effect of magnesium ammonium phosphate upon the Azotobacter. It was found that the addition of this compound to the soil plaques or the nitrogen-free agar medium used for the detection of the Azotobacter in soils, brought about a definite increase in the numbers of colonies appearing on the surface of the plates showing that it stimulated the growth of the bacteria. The reason for the stimulative action of the magnesium ammonium phosphate was traced to its content of available nitrogen, although, because of the slight solubility of this compound in water, only small amounts were present in the medium.

In a second experiment, the influence of different fertilizers or combinations of fertilizers upon the growth and activity of Azotobacter in typical samples of Clinton, Grundy and Clarion silt loams was determined. These soils represent a wide variety of soil conditions and, in addition, had been found to be generally lacking in Azotobacter.

The fertilizers used in these experiments were (a) lime to vary the pH of the samples, (b) oat straw to increase the organic matter content, (c) sodium nitrate to determine whether or not small amounts of nitrogen added to these soils would actually stimulate Azotobacter growth and (d) treble superphosphate to increase the amount of readily available phosphate.

The results showed that an addition of lime to these soils was essential for the prolonged growth of the bacteria and that an amount sufficient to raise the pH to near neutrality was all that was necessary to improve

[1] Original thesis submitted July, 1937. Doctoral thesis number 440.

the environment so that the bacteria would remain active for at least 9 months. The growth of corn, wheat or timothy on the Clinton silt loam or a Clarion fine sand treated with lime did not hinder the growth of the bacteria.

None of the individual treatments other than lime were essential for the growth of the bacteria in these soils. Indeed, except for oat straw, none of the treatments other than lime, regardless of their combination, seemed to exert any influence upon the bacteria. There was some evidence when lime was present, however, that other treatments may aid the growth of the bacteria. This was particularly true for the oat straw treatment in the case of the Clinton silt loam.

The limiting pH value for the growth of the Azotobacter in these soils was shown to approach a pH of 6.0 but it was pointed out that the actual limiting value for the different types probably varied with the general soil conditions. Thus, at the same pH, the Grundy silt loam presented a better medium for the growth and activity of the Azotobacter than did the Clinton silt loam. The results indicated that the higher organic matter content of the Grundy silt loam may account for this difference.

Having found that a active flora of the Azotobacter could be maintained in some acid Iowa soils by the addition of lime sufficient to raise the pH up to about the neutral point, inoculation experiments with the Azotobacter were conducted to determine whether or not sufficient nitrogen would be fixed to stimulate the growth of some typical Iowa crops. They were carried out in the greenhouse in triplicate with two soil types which responded to ntirogen fertilization, that is, Clinton silt loam and Clarion fine sand, and three Iowa crops, namely, corn, wheat and timothy.

The inoculation of the Clarion fine sand was completely without effect in altering either the dry weight or total nitrogen content of any of the crops. Tests of this soil for the presence of the Azotobacter during the course of the experiment showed that they were present and active when the soil had been treated with lime.

The inoculation of Clinton silt loam with Azotobacter was without effect in altering the dry weight and total nitrogen content of the corn and wheat grown upon it. The timothy, however, responded very definitely to the inoculation and a highly significant increase in both the dry weight and total nitrogen content of the crop was obtained. The average increase in the dry weight of the timothy due to the inoculation was about 39.5 per cent; for the total nitrogen content, an increase of about 26.6 per cent occurred. The Azotobacter were more active during the course of the experiment in the soils which had been treated with lime. The growth of the bacteria in the unlimed soils, however, was large enough that the response of the timothy to the inoculation did not differ significantly from that on the samples treated with lime.

These results indicate a need for more extended experiments upon the inoculation of soils with Azotobacter to test out a greater variety of plants on many different soils. When this is done, definite conclusions regarding the usefulness of the inoculation of soils with Azotobacter in our soil management practices may then be reached.

CHARACTERIZATION AND DIFFERENTIATION OF THE "INTERMEDIATES" OF THE COLON-AEROGENES GROUP OF BACTERIA[1]

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An investigation was made of the availability of the nitrogen of nucleic acid and certain of its degradation products for the coli-aerogenes group of bacteria in order to secure an approach, other than the carbohydrate studies, to the systematic and physiological relations of the group. It was hoped that such a study might throw light on the relations of the "intermediate group" to the genera Escherichia and Aerobacter.

Investigations were concerned primarily with the availability of the nitrogen of nucleic acid and its degradation products. At the same time these compounds were tested in an exploratory manner as to their carbon, or carbon and nitrogen availability, and as to the effect of moderate autoclaving. In addition, the effect of temperature and period of incubation was determined for the pyruvic acid fixation test as suggested by Reynolds (1935).

The compounds tested included: yeast nucleic acid, xanthine, adenine sulphate, uric acid, uracil, allantoin, hydantoin, and urea.

The basal test media consisted of: 0.5 per cent NaCl; 0.02 per cent $MgSO_4$; and 0.004 per cent brom-thymol-blue. To investigate the availability of nitrogen, dextrose, (0.2 per cent) was supplied as the carbon source, and the test compound (0.05 per cent) as the nitrogen source. To investigate the availability of carbon ammonium phosphate (0.2 per cent) was supplied as the nitrogen source, and the test compound (0.05 per cent) as the carbon source. To determine the simultaneous availability of carbon and nitrogen, the test compound (0.5 per cent) alone was employed. Two per cent by volume of phosphate buffer (pH 7.1) was added to all media; if this did not adjust the reaction to approximately pH 7.1, more of the necessary solution was added.

The constituents were dissolved in conductivity water at a temperature below boiling. All media were sterilized by filtration through Chamberlain candles. Inoculation consisted of a one mm. loop of a 24 hour broth culture. Incubation was for five days at 30° C. (temperature of the medium).

Availability of nitrogen was judged by two criteria: (1) acidification of the medium, as it was assumed that the dextrose would be attacked, thus creating an acid reaction, only if there were an available and adequate nitrogen source; and (2) vigor of growth.

The bacteria employed in the nitrogen availability studies included: 106 Escherichia; 39 *A. cloacae;* 31 *A. aerogenes,* indol +; 41 *A. aerogenes*, indol — ; 138 "intermediate" strains, 75 of which were contributed by workers outside this laboratory; and six strains which gave questionable VP reactions. This collection of cultures was considered representative of the groups in question.

[1] Original thesis submitted July, 1937. Doctoral thesis number 437.

The results of the fourth day of incubation showed that all strains tested utilized the nitrogen of adenine sulphate and xanthine. Since these compounds had no differential value, they were not considered further. The Aerobacter and VP questionable strains utilized the nitrogen of all other compounds tested, whereas a high percentage of the Escherichia utilized only the nitrogen of uracil, and the "intermediates" only the nitrogen of urea.

Vigor of growth was employed as the criterion of availability of the carbon, or both carbon and nitrogen, in the compounds studied. Observations with a limited number of strains indicated that of the compounds tested as carbon, or carbon and nitrogen sources, all but adenine sulphate supported a vigorous growth of *A. aerogenes,* but not *Escherichia, A. cloacae,* or "intermediate" strains. The adenine served as an available source of carbon, but not carbon and nitrogen, for *A. aerogenes* strains.

The test compounds (nuclei acid, uracil, allantoin, hydantoin, urea) were subjected to moderate autoclaving at 15 pounds for 15 minutes and the media cooled immediately. Subsequent inoculation with a limited number of organisms showed that of the compounds as nitrogen sources, that nucleic acid and uracil were not affected appreciably, but that allantoin, hydantoin, and urea were apparently modified by the heat treatment.

Investigations were carried out to test the possibilities of pyruvic acid fixation in a glycerol-peptone-bisulphite medium as a means of differentiating the "intermediate" strains from those belonging to the genera Escherichia and Aerobacter. Reynold's medium (1935) and the nitroprusside test for pyruvic acid (Simon and Piaux) were employed. It was found that both time and temperature had a decided effect on this test, and that due to the variability of results for the same organisms from day to day the establishment of standard conditions for a routine test would be difficult to attain.

A study of the individual reactions of the "intermediate" strains showed that: 83 per cent produced hydrogen sulphide (Vaughn and Levine medium); 94 per cent failed to produce indol from tryptophane; and that 95 per cent attacked cellobiose with production of either acid or acid and gas. A transfer of the original type species, *Citrobacter Freundii,* gave reactions typical of those given by a large proportion of the "intermediate" strains.

The VP questionable strains were allocated to the genus Aerobacter. The genus Citrobacter was accorded generic ranking with the genera Escherichia and Aerobacter.

REFERENCES

REYNOLDS, H.
1935. The dissimilation of carbohydrates by the colon-aerogenes group of bacteria. Unpublished thesis. Library, Iowa State College, Ames, Iowa.

SIMON, L. G., and L. PIAUX
1924. Sur le characterisation et la dosage le petites quantities d'acide pyruvique. Bul. de la Soc. Chem. Biol. 6:477-486.

VAUGHN, R., and M. LEVINE
1936. Hydrogen sulphide production as a differential test in the colon group. Jour. Bact. 32:65-73.

BACTERIOPHAGE SPECIFICITY FOR COLIFORM AND RELATED BACTERIA[1]

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The specificity of bacteriophages for inducing lysis of different bacteria has not been extensively studied. It is generally recognized, however, that a certain degree of specificity does exist. d'Herelle (1) believes that some phages, when tested against members of the colon-typhoid group of bacteria, may attack only certain closely-related strains, but that other phages may attack bacteria in several different genera. Beard (2), using various methods, was unable to produce any adaptation of bacteriophages. Burnet and McKie (3) made extensive studies on the classification of phages, but have done only a limited amount of work on specificity. Evans (4) has employed bacteriophage to differentiate Streptococci. Krueger (5) reviewed some of the important contributions regarding bacteriophage.

The bacterial cultures against which bacteriophages were isolated and purified in this study include strains of Escherichia, "Intermediates," Aerobacter, Proteus, Salmonella, Eberthella, Shigella, Alkaligenes, Klebsiella, Pseudomonas, Flavobacterium, Serratia, and Achromobacter. In addition to the different strains for which phages against the foregoing bacteria were isolated, a large number of additional strains from the genera listed, particularly the coli-aerogenes group, were employed for studying the specificity of the bacteriophages.

The raw material for the isolation of the bacteriophages was sewage from the effluent of the Imhoff tank of the Ames sewage disposal plant and the effluent from the trickling filters of a packing house at Mason City, Iowa. For each bacteriophage isolated a preliminary enrichment tube was first prepared consisting of 10.0 cc. of broth, 1.0 cc. of the sewage and a light inoculum of the bacterial culture against which an homologous phage was desired. The enrichment tubes were incubated for 24 hours at 30° C. after filtration through sterile Chamberland-Pasteur L_3 filter candles and were then ready for testing and purification by plating procedures.

Petri dishes were prepared containing 15.0 to 20.0 cc. of nutrient agar of the following composition: 1.5 per cent Bacto peptone, 0.3 per cent beef extract, and 0.1 per cent agar. The plates were inoculated at the center with some bacteria from agar slants incubated 18 hours. The inoculum was smeared over the surface of the agar with a sterile, bent, glass smearing rod.

For inoculating the bacteriophages a 3.0 mm. platinum loop bent at an angle of 120° was used. One loopful of a phage filtrate was streaked successively across each petri dish prepared as described above. After incubation for 18 hours at 30° C. isolated phage plaques, usually of different size, appearance, and intensity, could be observed on the plates.

[1] Original thesis submitted March, 1938. Doctoral thesis number 457.

Suitable, isolated plaques, together with some bacterial culture, were picked from each plate, emulsified in 10 cc. of broth, incubated, filtered, and replated, as just described. This procedure was repeated at least six times. Forty-three "pure" races of phage were obtained by this means. Twenty-eight of the 43 phages isolated were against coli-aerogenes strains.

In making specificity tests agar plates were prepared, inoculated, and smeared as before. Two phages were streaked on each plate. Each "pure" race of a phage employed for tests was inoculated onto a plate smeared with the culture to be tested for lysis. The phage was inoculated by making one streak about 2 inches long with the platinum loop. The plates were incubated for 18 hours and the results recorded. The test was considered to indicate positive lysis if no bacterial growth, or occasionally only partial bacterial growth, occurred in the area of the phage inoculum.

Two main series of specificity experiments were performed. In the first series 43 phages were tested against 94 coliform and related bacteria. In the second experiment 14 phages isolated against coliform bacteria were tested against 144 coliform organisms. The results indicate that:

There was a marked tendency to specificity of phages for the two main divisions of the coli-aerogenes group, as the following tabulation shows:

Cultures Tested	Phages Employed	Total No. of Tests	No. of Positive Tests	Percentage Lysed
20 Escherichia	10 "Escherichia	200	47	23.5
20 Aerobacter	8 "Aerobacter"	160	47	29.4
20 Escherichia	8 "Aerobacter"	160	0	0.0
20 Aerobacter	10 "Escherichia	200	1	0.5

The "Intermediates" show a moderate tendency to specificity of phages in the coli-aerogenes group, as the following indicates:

Cultures Tested	Phages Employed	Total No. of Tests	No. of Positive Tests	Percentage Lysed
20 "Intermediate"	8 "Intermediate"	160	46	28.7
20 Escherichia	8 "Intermediate"	160	13	8.1
20 "Intermediate"	10 "Escherichia"	200	11	5.5
20 Aerobacter	8 "Intermediate"	160	2	1.2
20 "Intermediate"	10 "Aerobacter"	200	2	1.0

The above figures also indicate, that on the basis of these bacteriophage tests, the "Intermediates" are more closely related to the genus Escherichia than to the genus Aerobacter.

No evidence of bacteriophage specificity was observed within the major sub-groups of the coli-aerogenes group of bacteria. There was no correlation between sucrose fermentation and susceptibility to lysis by

phage among the strains of the genus Escherichia or the "Intermediates," and no correlation between ability to ferment starch or to form indol from tryptophane and susceptibility to lysis by phage among the strains of the genus Aerobacter.

Genera for which phages appear to be specific include Proteus, Flavobacterium, Serratia, and Klebsiella. The remaining phages in this study (active against bacteria in such genera as Escherichia, Aerobacter, "Intermediates," Salmonella, Eberthella, Shigella, Alkaligenes, Pseudomonas, and Achromobacter) did not lyse members of the first four genera mentioned, except that a phage active against *Achromobacter lipolyticus* also lysed a strain of *Serratia indica.*

A reddish-orange, lactose-fermenting organism studied was more closely related to the coli-aerogenes bacteria than to the genus Serratia on the basis of phage tests. The organism was lysed by 2 Escherichia phages and 1 "Intermediate" phage, but no other phages, including 2 phages active against strains in the genus Serratia.

Some phages are limited in activity, attacking only coli-aerogenes strains, while others are active against a wider range of organisms (polyvalent phages), causing lysis of coli-aerogenes strains and also organisms associated with intestinal infections.

Many different races of phage appear to exist, as most of the 43 phages studied show different lytic specificities.

LITERATURE CITED

BEARD, P. J.
1931. Bacteriophage adaptation. J. Infect. Dis. **49**:367-398.

BURNET, F. M., and M. MCKIE
1933. The classification of the coli-dysentery bacteriophages. I. The differentration by Bail's methods of phages lysing a typical B. coli strain. J. Path. and Bact. **36**:299-306.

D'HERELLE, FELIX
1926. The Bacteriophage and Its Behavior. Williams and Wilkins, Baltimore.

EVANS, A. C.
1936. Studies on hemolytic streptococci. I. Methods of classification. J. Bact. **31**:423-437.

KRUEGER, A. P.
1936. The nature of bacteriophage and its mode of action. Physiological Rev. **16**:129-172.

THE PRINCIPLE OF THE ARCHIMEDEAN SCREW IN CHEMICAL ENGINEERING[1]

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The theory of the Archimedean screw is developed, and the possibility of its application as a pump in chemical engineering, and the application of the principle by which it elevates liquids, to various chemical engineering operations, are investigated.

From the point of view of the chemical engineer the Archimedean screw has a number of characteristics in its favor. It is completely valveless, essentially simple, and may be constructed of corrosion resisting materials. Although there are several modifications of the screw, the form which is considered in the investigation consists of a central cylindrical core on which are wound one or more vertical spiral flights, and an external cylindrical casing attached to the flights. The device is mounted at an angle with the horizontal so that the lower end dips into the liquid to be elevated, and is rotated about its axis.

The simple Archimedean screw is open to the objections that it is extremely sensitive to the external liquid level, and unless conditions are very carefully controlled, performance is apt to be erratic and the efficiency low. It is shown, however, by an examination of the theory of the Archimedean screw, that the low volumetric efficiency of the simple screw is due to the fact that as the screw rotates and successive slugs of liquid are picked up a partial vacuum is created in the air space between successive slugs. This action is cumulative, and eventually is sufficient to draw some or all of the liquid that has been carried part way up the screw back down the tube. This condition may readily be corrected, and high volumetric efficiency be obtained, by venting the air spaces between the liquid slugs to the atmosphere and thus equalizing the pressure throughout the screw.

The desired venting of the screw may be accomplished by any of several methods. Prior to this investigation, it was known that if the screw were operated in such a manner that the cross section of the spiral tubes was at no point completely filled with liquid, thus insuring a continuous air passageway through the screw, or if slots were cut in the core of the screw just below and following the lower surfaces of the several spiral flights, thus making it possible for air to flow in through the hollow core and balance the pressure, high volumetric efficiency was obtained. In either case, however, the quantity of liquid handled by a given screw was small. It is shown that much more liquid may be lifted if the screw is vented by a series of venting tubes in the first few turns of each of the spiral tubes, the venting tubes being inserted in holes in the casing and extending far enough up the casing parallel to the axis of the screw so that liquid will not flow out the open upper end. Or a slot may be cut in

[1] Original thesis submitted June, 1938. Doctoral thesis number 462.

the casing and the screw protected from loss of liquid through the slot by an external spiral sheet wrapped around and close to the casing, the lower edge of the sheet being attached to the lower edge of the slot. The quantity of liquid handled by a vented Archimedean screw is almost exactly the theoretical.

The capacity of the Archimedean screw as a pump is a function of the several variables in its design and the relationships of theoretical capacity to these variables are developed. The quantity of liquid handled by a vented screw, even when the lower end is completely immersed, varies directly as the speed of rotation. The capacity decreases with increasing angle of inclination, and is a function of the pitch of the flights. For each angle of inclination there is a specific pitch for maximum capacity, the optimum pitch decreasing with increasing inclination of the screw. The capacity is also a function of the ratio of core diameter to casing diameter, but the optimum ratio seems to be constant at one-half. The capacity of the screw is increased by the introduction of additional flights, but beyond four or five the slight gain in capacity does not warrant further addition.

The mathematical expression for the exact theoretical capacity of the Arichamedean screw as a pump in terms of its design is quite involved. A graphical method by which the theoretical capacity may be closely approximated is developed. The graphical method is rather laborious, and a quicker, though under certain conditions somewhat less exact method, is developed on the assumption that the length of the wetted center line of the spiral tubes of the Archimedean screw is proportional to the volume of liquid in the tubes. By the use of the latter method the theoretical capacities of a number of screws were calculated and the above-mentioned relationships were developed.

Several small Archimedean screws were set up and tested, and the theories developed were confirmed.

If the height of the external spiral sheet, or "sidewall," above the flight of the vented screw mentioned above, be reduced as the external sidewall winds around the screw, liquid from within the screw will spill over the upper edge of the sidewall. If the reduction in height of the sidewall is gradual, the liquid will spill over its upper edge continuously as the screw rotates. It is shown that by attaching a continuous sheet fin to the upper edge of the sidewall the falling liquid may be distributed over a considerable area. The contour of the sidewall may be easily designed for any manner or rate of distribution of liquid over the fin.

A device such as outlined above would have the effect of lifting liquid through a small distance and at the same time distributing it over a large area, thus facilitating heat or gas transfer through the gas-liquid interface. A number of chemical engineering operations, notably aeration, gas scrubbing or absorption, stripping, heat transfer from gas to liquid or liquid to gas, and the like, are dependent upon obtaining as large an area as possible per unit volume of liquid, and a device of the type described should be applicable. Two such experimental "aerators" were set up and tested for rate of gas absorption and heat transfer, and the results, although inconclusive as yet, are sufficiently encouraging to warrant an extended investigation of the possibilities of the device.

PASSAGE-TIME OF VARIOUS TYPES OF NORMAL AND POISONED FOODS THROUGH THE ALIMENTARY TRACT OF THE COCKROACH PERIPLANETA AMERICANA LINN[1]

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Investigations were conducted to determine rates of passage of some normal and poisoned foods through the alimentary tract of the cockroach, *Periplaneta americana* Linn. Approximately 700 adults and last instar nymphs were dissected to determine progress of dyed material along the digestive canal at various intervals after ingestion; about 900 were tested for total passage-time (designated as egestion time) by means of mechanical excrementometers devised for this purpose. These mechanisms are figured and described in detail. All insects were starved for 48 hours before beginning tests. Only normal active specimens were used. Experimental animals were permitted access to water throughout the trials.

Banana paste or commercial Purina chow formed the base of all test meals used. Five per cent powdered carmine dye was added to each preparation to render test material identifiable in the digestive tract and in the fecal pellets. All test meals were slightly sweetened by addition of 1 per cent finely pulverized cane sugar. Passage-times of the 13 following test meals were determined: banana paste, desiccated banana, banana-barium sulphate, banana-sodium fluoride, banana-phenothizine, banana-arsenate of lead, banana-astringent arsenate of lead, banana-arsenic trioxide, banana-rotenone, Purina powder, Purina-barium sulphate, Purina-sodium fluoride, and Purina-phenothiazine.

Figures are included to illustrate average rates of progress of these test meals and tables list egestion time records of all populations used.

First ingested portions of banana paste pass through the stomodaeum in less than one-half hour, complete passage along the mesenteron and enter the pylorus in 2.5 to 3 hours, require from 5 to 6 hours in the anterior intestine, and remain 10 to 12 hours in the rectum. It seems quite evident that portions of the anterior intestine and rectal epithelium function in absorbing considerable liquid from semi-fluid material present in the lumen of the proctodaeum. Total passage-times for banana paste residues averaged 20.22 ± 6.9 hours. Repeated trials with the same test insect demonstrated considerable inconsistency in individual egestion times.

Rate of progress of Purina powder is quite similar to movement of banana paste along the stomodaeum and mesenteron. From 3.5 to 4 hours are required for passage of the Purina residue through the anterior intestine; rectal retention time is normally about 14 hours. Mean egestion time for indigestible residue of this material was 21.45 ± 6.93 hours.

Mean egestion time of desiccated banana was 13.48 ± 3.79 hours. Average elimination period of banana-barium sulphate residua was 15.58 ± 6.47 hours. Velocity of passage along the anterior intestine was

[1] Original thesis submitted July, 1937. Doctoral thesis number 443.

considerably increased. Mean egestion time for Purina-barium sulphate waste was 20.03 ± 7.83 hours. Sodium fluoride caused great decreases in rate of movement through the anterior intestine. Mean egestion times for banana-sodium fluoride and Purina-sodium fluoride were 30.99 ± 11.75 hours and 28.67 ± 9.21 hours, respectively. Phenothiazine with banana paste produced a decrease of about 2 hours in the period required in the anterior intestine. Egestion times for residua of this material averaged 18.05 ± 7.78 hours. Mean total passage-time for Purina-phenothiazine waste was 21.42 ± 7.48 hours.

Banana-arsenate of lead preparations (with or without astringent) showed somewhat greater velocity of movement through the anterior intestine. Mean egestion records for lead arsenate and astringent lead arsenaet with banana paste were 18.08 ± 8.66 hours and 17.57 ± 7.09 hours. respectively. Arsenic trioxide with banana paste caused noticeable destruction of ventricular tissue and greatly decreased rate of progress through the anterior portion of the proctodaeum. Distribution of this material in the mesenteron was irregular as greater concentrations usually occurred at the cardiac and pyloric ends. Mean time of evacuation of first portions of this food-poison residuum was 28.91 ± 5.81 hours. Rotenone with banana caused a decrease of 2.5 hours in time necessary in the anterior intestine. Mean egestion time for indigestible residue of this material was 18.24 ± 5.97 hours.

Records of mortality produced by food-poison mixtures during a 50 hour observation period are listed. Highest kill (53 per cent) was recorded with banana-arsenic trioxide.

Normal velocities of food movement in man and this insect are physiologically comparable. In man food material begins to leave the stomach immediately after ingestion, passes rapidly through duodenum and jejunum, moves progressively slower in the ileum as the ileocaecal valve is approached, is retained in the colon from 12 to 14 hours, and is normally evacuated from 16 to 24 hours after entering the stomach. A decrease in velocity of food movement along the alimentary tract of the roach is evident in the vicinities of the mesentero-proctodael junction and the intestino-rectal constriction. Comparison of these relationships does not infer morphological similarity.

In addition to changes in rate of progress produced by variations in chemical and physical characters of the food, the following factors were investigated to determine their effects on velocity of food movement: sex, stage of development, amount of material ingested, length of starvation period before feeding, size of previous meal, excitement or undue disturbance, and amount of general muscular activity.

Sex and stage of development (limited to adults and nymphs in last instar) were not found to influence velocity of food movement. The following factors were found to increase rate of food progress: ingestion of larger amounts of unpoisoned preparation (measured by degree of crop distention), undue excitement or fright, longer periods without food before testing, and general increase in muscular activity. Decreases in velocity of movement of sodium fluoride and arsenic trioxide mixtures were correlated with the amount of food-poison ingested. Velocity of progress was decreased by the presence of large amounts of a previous meal in the stomodaeum.

STUDIES ON LACTOBACILLUS CULTURES THAT ACTIVELY COAGULATE MILK[1]

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The primary object of the work herein reported was to determine the general relationships of cultures of Lactobacilli that actively coagulate litmus milk with reduction of the litmus and the formation of a smooth curd. Most of the cultures were isolated from dairy products and materials such as ensilage, corn stover and feces from calves, dogs, rats and infants. Cultures of *L. acidophilus* and *L. bulgaricus* were obtained from various laboratories for comparative purposes. A total of 36 cultures was studied, particularly from the standpoint of their action in milk because of the importance of the organisms in dairy products.

In a comparison of the value of various solid media for isolating Lactobacilli, it was found that tomato juice agar gave higher and more uniform counts than beef infusion agar plus 1 per cent lactose, cabbage agar, casein digest agar or whey agar; the results obtained with beef infusion agar plus lactose were strikingly lower than the counts obtained with the other media. The isolation of Lactobacilli from dairy products was facilitated by incubating the material being examined in milk until the microflora showed a predominance of characteristic rod forms, and then plating on tomato juice agar. Nutrient broth containing 2.0 per cent dextrose and 5.0 per cent N/10 acetic acid was useful as an enrichment medium in isolating Lactobacilli from fecal material.

Appreciable quantities of total acid, volatile acid and acetylmethylcarbinol plus diacetyl were produced in milk by most of the 36 cultures of Lactobacilli studied but the amounts of these materials produced by the different cultures varied greatly and there was no close correlation between the amounts of total acid and the amounts of volatile acid or acetylmethylcarbinol plus diacetyl produced by a culture. The addition of citric acid or acetaldehyde to milk cultures did not significantly influence the production of volatile acid or of acetylmethylcarbinol plus diacetyl by the organisms. Low concentrations of the acetaldehyde resulted in slight increases in the amounts of acetylmethylcarbinol plus diacetyl produced while higher concentrations appeared to be toxic to the organisms.

Most of the cultures studied produced appreciable quantities of carbon dioxide in skim milk; the values were generally lower when peptone was added to the milk. More carbon dioxide was produced at 37° C. than at 21° C. during incubation for 15 days.

The fermentation reactions obtained with the Lactobacilli studied were too variable to be used as criteria for the separation of the cultures into species. Dextrin, dextrose and lactose were the carbohydrates most commonly attacked. None of the organisms that grew on nile-blue sulfate agar hydrolyzed butter fat.

[1] Original thesis submitted June, 1936. Doctoral thesis number 386.

The isomeric form of lactic acid produced was determined with 30 of the cultures. The form of acid varied from pure active to nearly pure inactive and was usually dextro-rotatory. There was apparently no relation between the types of lactic acid produced and the sources of the organisms. Most of the cultures of Lactobacilli were able to break down the proteins in milk as indicated by increases in amino nitrogen content during incubation at 37° C. for 8 weeks. Larger amounts of amino nitrogen were produced when the acid developed was partially neutralized with calcium carbonate.

The addition of 0.13, 0.7 or 5.0 per cent of deuterium oxide to whey broth cultures of Lactobacilli had no influence on the morphology or rate of multiplication of the organisms.

Evidence was obtained which indicated that the growth temperatures may be useful in classifying the Lactobacilli. All the organisms grew well and coagulated milk at 37° C. but the results obtained at the other temperatures were variable. The *L. acidophilus* and *L. bulgaricus* cultures obtained from various research laboratories and the cultures isolated from fecal material grew well at 37° and 45° C. but not at 10° or 21° C. In general, the Lactobacilli from raw milk, Swiss cheese, Cheddar cheese and feed grew at 21° C. but not at 45° C.; a few of these organisms also grew at 10° C.

None of the Lactobacilli produced pronounced flavors in milk other than acid, which varied from mild and desirable to sharp, high acid and undesirable. The odor produced in milk by the Lactobacilli suggested acid and varied from none to very strong. Cultures that produced a clean, mild acid flavor usually gave a clean, desirable acid odor, while the cultures that produced an unclean, flat acid flavor gave an unclean odor. There appeared to be no relation between the sources of the organisms and the flavors and odors produced in milk.

In isolating the Lactobacilli, organisms were encountered which resembled Lactobacilli very closely but which did not coagulate milk rapidly. This suggests that not all Lactobacilli coagulate milk rapidly or that there is a group of closely related organisms which do not grow well in milk.

THE RING STRUCTURE IN SOME DERIVATIVES OF SORBOSE[1]

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The assignment of ring configuration is of first importance in developing the chemistry of sugar derivatives. With the exceptions of diacetone sorbose (1) and the open chain pentacetate (2, 3) the ring structures for *l*-sorbose and its derivatives have, up to this time, remained undetermined.

Since α-methyl-*l*-sorboside was one of the earliest known derivatives of sorbose, and since the glycosides are important central compounds in the sugar series, the determination of ring configuration for α-methyl-*l*-sorboside was undertaken. α-Methyl-*l*-sorboside was methylated to a pentamethyl derivative, which was hydrolyzed to a tetramethyl-*l*-sorbose (4). Nitric acid oxidation of this latter compound resulted in the production of dextro dimethoxysuccinic acid in good yield. The presence of this acid was established through the preparaton of its amide and methylamide. These are well-defined crystalline derivatives prepared by Haworth (5) as reference compounds in the sugar series. The isolation of dextro dimethoxysuccinic acid asserted the methylation of carbon atoms four and five in the tetramethyl-*l*-sorbose, since no other adjacent carbon atoms could give rise to an acid of this configuration. Hence, the lactol ring must have engaged either carbon atom three or carbon atom six in the tetramethyl sorbose. As ethylene oxide rings are not known to form under the conditions employed for glycoside formation, the possible existence of such a ring was not considered. This left only a six membered, or normal pyranoid ring structure, to be assumed for the tetramethyl-*l*-sorbose and, hence, also for α-methyl-*l*-sorboside and its derivatives.

When *l*-sorbose was treated with dry ethanol containing one per cent hydrogen chloride, α-ethyl-*l*-sorboside was produced. This compound crystallized as fine colorless needles having a melting point of 116° and a specific optical rotation in water of $[\alpha]_D^{26} - 73.9°$. Acetylation of this substance produced α-ethyl-*l*-sorboside tetraacetate having a melting point of 75° and a specific optical rotation in chloroform of $[\alpha]_D^{26} - 54.6°$. This α-ethyl-*l*-sorboside tetraacetate was identical with that obtained through the ethylation of sorbose tetraacetate. Thus, the ring structure of α-ethyl-*l*-sorboside must be the same as that in sorbose tetraacetate. The structure of sorbose tetraacetate as 1,3,4,5-tetraacetyl sorbose can be established through the fact that acetylation of α-methyl-*l*-sorbopyranoside, whose configuration was proven as indicated above, yields an α-methyl-*l*-sorboside tetraacetate identical with the compound obtained through the methylation of sorbose tetraacetate. This series of reactions indicated a normal pyranoid ring structure for α-ethyl-*l*-sorboside and its tetraacetate.

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In comparing the rates of formation of α-methyl-*l*-sorbopyranoside and α-ethyl-*l*-sorbopyranoside it was found that the reactions were essentially complete in four hours, when the *l*-sorbose was dissolved in either methanol or ethanol containing one per cent hydrogen chloride and the mixture allowed to stand at room temperature.

The rates of hydrolysis for α-methyl-*l*-sorbopyranoside and α-ethyl-*l*-sorbopyranoside were also found to be almost identical. Dissolved in 0.015 normal hydrochloric acid both glycosides were hydrolyzed in about thirty days when kept at a temperature of 30°. It was interesting to note in this connection that these rates of hydrolysis did not differ widely from the rate found for methyl-*d*-fructofuranoside (6).

Calcium chloride was known to form crystalline addition compounds with several sugars. The preparation of a calcium chloride compound of *l*-sorbose was undertaken in the hope of obtaining a compound of sorbose in either of its alpha or beta stereoisomeric forms. When *l*-sorbose and calcium chloride in equal molar proportions were dissolved in water and the solution allowed to evaporate slowly, fine crystals of a calcium chloride addition compound of α-*l*-sorbose were obtained. After purification the crystals showed a melting point of 159° (Corr.) and a specific optical rotation in water of $[\alpha]_D^{26} - 24.2°$. This compound exhibited a rapid but small upward rotation. In fifteen minutes the value became constant at $[\alpha]_D^{26} - 23.9°$. The compound gave the following formula on analysis: $C_6H_{12}O_6 \cdot CaCl_2 \cdot 2H_2O$. On acetylating the addition compound by the general procedure for acetylation there was produced in good yield keto sorbose pentaacetate. Hence, the property of *l*-sorbose to form preferentially an open-chain pentaacetate is also exhibited by the calcium chloride addition compound. Acetylation, using the special method designed to give sorbose tetraacetate, produced this tetraacetate in good yield. These facts indicate that calcium chloride does not stabilize sorbose in a new form.

By means of hydrogenation and acetylation followed by the subsequent isolation of the hexaacetate of *d*-sorbitol and *l*-iditol, Cramer and Pacsu (3) have proven that the common pentaacetate of *l*-sorbose is a derivative of the open-chain sugar. This conclusion might have further been substantiated if it could have been shown that sorbose pentaacetate were capable of mercaptalization to yield *l*-sorbose ethyl thioacetal pentaacetate. Following the method advanced by Wolfrom (7) for the mercaptalization of fructose, the mercaptalization of keto sorbose pentaacetate was carried out. The product obtained was a colorless sirup which could not be crystallized. The sirup showed no reducing properties when tested wtih Fehling's solution. The sirup had a refractive index of N_D^{24} 1.5030 and specific optical rotation in chloroform of $[\alpha]_D^{27} - 12.2°$. The substance distilled in a high vacuum and at a bath temperature of 200° to produce a sirup having a refractive index of N_D^{24} 1.5050 and a specific optical rotation in chloroform of $[\alpha]_D^{30} - 13.1°$. The distillate was light brown in color showing that a slight decomposition had occurred during the distillation. Neither the original sirup nor the liquid distillate showed any evidence of mutarotation.

LITERATURE CITED

(1) Reichstein and Grussner
1934. Helv. Chim. Acta, **17**, 311.

(2) Schluback and Vorweck
1933. Ber., **66**, 1251.

(3) Cramer and Pacsu
1937. J. Am. Chem. Soc., **59**, 1467.

(4) Arragon
1936. Bull. soc. chim. biol., **18**, 1336.

(5) Haworth and Jones
1927. J. Chem| Soc., 2349.

(6) Menzies
1922 *Ibid.* ,2238.

(7) Wolfrom and Thompson
1934. J. Am. Chem. Soc., **56**, 880.

STUDIES ON THE PHYSICAL-CHEMICAL PROPERTIES OF HONEY[1]

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DeBoer and Kniphorst (1932) found that a heather honey exhibited the gel/sol transformation brought about by stirring which has been termed thixotropy by Freundlich (1928). Scott Blair (1935) observed the phenomenon in another heather honey, as did Pryce-Jones (1936a). Honeys from sources other than heather were found to be non-thixotropic. Convincing evidence has been advanced by Pryce-Jones to show that colloidal materials in heather honey are responsible for its unique behavior.

Paine, Gertler and Lothrop (1934) have investigated a second property of honey, closely related to thixotropy, that of anomalous viscosity. The apparent viscosity of buckwheat honey was found to vary with the shearing force involved in the measurements. The anomalous behavior disappeared when the colloidal material was removed by ultrafiltration.

The accurate measurement of thixotropy is difficult; the only means available, in many cases, of following the gel/sol transformation is to make observations of the apparent viscosity before and after stirring. Pryce-Jones (1936b), however, has claimed that all viscometric methods so far proposed are subject to error. There are two obstacles inherent. First, a certain amount of stirring is involved in the measurement of the viscosity. This tends to lower the apparent viscosity and the lowering will be different for various observations. Second, viscosity determinations on the sol and gel forms, which may differ widely in apparent viscosity, are not readily made at the same shearing force. Since the apparent viscosity of each form is a function of the shearing force, comparisons of the apparent viscosities have little meaning unless this variable is held constant.

The falling sphere viscometer was chosen for this research since it has found wide use in such studies and because it seemed to offer certain advantages over other instruments. The stirring actions of the smallest spheres is practically negligible and by proceeding from small to large spheres in the measurements it is possible to obtain the curve, apparent viscosity against shearing force, for the undisturbed gel. The same curve is readily obtained for the sol form, on which stirring has no effect, and comparison at the same shearing force can be made from the two curves. A standard shearing force of zero was selected at which comparisons were made. Apparent viscosities η, were calculated from the velocity of the sphere by the following equation,

$$\eta = \frac{2(d_2 - d_1)\, g r^2 f_w}{9V} \qquad (1)$$

[1] Original thesis submitted December, 1937. Doctoral thesis number 451.

This is the Stokes' law equation multiplied by fw, the wall correction factor of Faxen (1922). Apparent viscosities were plotted against sphere radius and an extrapolation made to zero sphere size, that is, to zero shearing force. The number of observations made in fixing the curve for the gel form was limited as much as possible to avoid stirring. The honey was then stirred with a rotary stirrer and a second series of observations was made. The numerical value of the thixotropy was taken as the change in apparent viscosity in poises, at zero shearing force, which occurred on stirring. Since thixotropy changes with time and since the rate of change depends on the size of the containing vessel, it was necessary to fix arbitrary conditions under which the thixotropy of the honey was measured. The honey was heated for one hour at 60°, poured into a fall cylinder of 2.2 centimeters radius and allowed to cool to room temperature. The cylinder was then placed in the thermostat and the honey was maintained at 25° for 48 hours, at which time the measurements were made. The thixotropy of a number of Iowa honeys was measured and found to vary from 3 to 15 poises.

In order to rate the anomalous viscosity it was necessary to find the shearing force involved in each measurement. The equation of Lamb (1906) has heretofore been used by viscometrists to calculate the shearing force in the falling sphere viscometer. This equation is

$$F = \frac{3\eta V}{2r} \qquad (2)$$

where η is the apparent viscosity, V the velocity of the sphere and r its radius. Preliminary work with honey, however, indicated equation (2) to be incorrect. An equation for the same purpose has recently been advanced by Pasynskii and Rabinovich (1934). This is

$$F = \frac{\eta V}{R - r} \qquad (3)$$

where R is the radius of the fall cylinder and the other symbols have their previous meanings. Equation (3) gave indications of being valid. Honey, however, is a liquid definitely unsuited to use as a test liquid to decide between the two equations. For this purpose data are required which have been obtained with a variety of fall cylinders and spheres; thixotropic setting is most pronounced in a small vessel and a honey after standing in several fall cylinders would not be in a comparable state in each. The data available in the literature were considered and those of Sheppard (1917) on a nitrocellulose solution were chosen. The equation of Pasynskii and Rabinovich was found to give an excellent representation of the data, while that of Lamb did not. It is felt that the equation of Pasynskii and Rabinovich, apparently not available outside of a Russian publication until recently, is an important contribution to the theory of the falling sphere viscometer; it places this viscometer on an equal footing with capillary and rotating cylinder instruments for the study of anomalous systems. A further advantage of equation (3) is that data from the falling sphere viscometer and the capillary tube instruments on

plottng fall on a common apparent viscosity-shear force curve. This was not necessarily to be expected.

In several honeys the apparent fluidity, ϕ, was found to increase with the shearing force, F, according to the Bingham-Williamson equation,

$$\phi = a - b\ F \tag{4}$$

wheer a and b are constants. This equation is discussed in detail by Bingham (1928). In one honey, however, there was found a superimposed tendency to behave in an opposite manner, that is, the fluidity decreased with increasing shear force. This seemed to be the *elastic recoil* observed by Pryce-Jones (1936a), as far as such a property could be identified in a dissimilar instrument. The possibility of the presence of *elastic recoil* makes the rating of the anomalous viscosity uncertain. It was therefore decided not to attempt a numerical rating of this property. It is of interest, however, that each of the honeys observed showed an apparent viscosity which varied with the shearing force.

The honeys were analyzed for the water content by the refractometric method of Fulmer, Bosch, Park and Buchanan (1934) and the nitrogen content was determined. The pectin content was estimated by a modification of the method of Carré and Haynes (1922). A preliminary acidification with acetic acid, boiling and filtering was introduced to prevent the precipitation and weighing of protein as pectin. The volume of protein precipitated from 5 grams of diluted honey by phosphotungstic acid was determined. While Phyce-Jones found thixotropy only in honeys giving 30 cc. of precipitate, each of the honeys observed was definitely thixotropic and the volume of precipitate was never more than 0.3 cc. The lack of agreement is probably only apparent, since the heather honeys investigated by Pryce-Jones have anywhere from 200 to 2,000 times as much thixotropy as the honeys dealt with here.

Other investigators have found a general correlation between thixotropy and the colloidal content of the honey. The heather honey, for which thixotropy had been reported, contained up to 6 per cent of such materials. The colloidal content of the honeys studied in this thesis was less than 1 per cent and the observed thixotropy was much lower than that observed in heather honey. It is not surprising, therefore, that no definite relationship was found between the colloidal content and thixotropy. However, an invert sugar solution did not show thixotropy. It may be inferred, therefore, that the low thixotropy of the honey is associated with the colloidal material present.

LITERATURE CITED

BINGHAM, E. C.
1928. Colloid types. Colloid Symposium Monograph 5:219-228.

CARRÉ, M. H., and D. HAYNES
1922. The estimation of pectin as calcium pectate and the application of this method to the determination of the soluble pectin in apples. Biochem. J. **16**:60-69.

DEBOER, H. W. and L. C. E. KNIPHORST
1932. Thixotropie van Heidehonig. Chem. Weekblad **29**:526-534.

FAXEN, H.
1922. Die Bewegung einer starren Kugel längs der Achse eines mit zäher Flussigkeit gefüllten Rohres. Arkiv Mat. Rstron. Fysik 17, No. 27, 1-28.

FREUNDLICH, H.
1928. Ueber thixotropie. Kolloid-Z. 46:289-299.

FULMER, E. I., W. BOSCH, O. W. PARK, and J. H. BUCHANAN
1934. The analysis of the water content of honey by use of the refractometer. Am. Bee J. 12:208-212.

LAMB, H.
1906. Hydrodynamics. University Press, Cambridge.

PAINE, H. S., S. I. GERTLER, and R. E. LOTHROP
1934. Colloidal constituents of honey. Ind. Eng. Chem. 26:73-81.

PASYNSKI, A. G., and A. I. RABINOVICH
1934. The viscometry of highly viscous lyophilic colloids. II. Application of viscometers based on Stokes' law. (Translated title.) Zhur. Fiz. Khim. Leningr.) 5:521-533.

PRYCE-JONES, J.
1936a. Thixotropy and elastic-recoil in heather honey. Bee World 17:89-94.

PRYCE-JONES, J.
1936b. Some fundamental aspects of thixotropy. J. Oil and Colour Chem. Assoc. 19:295-337.

SCOTT BLAIR, G. W.
1935. The thixotropy of heather honey. J. Phys. Chem. 39:213-219.

SHEPPARD, S. E.
1917. The measurement of the absolute viscosity of very viscous media. Ind. Eng. Chem. 9:523-527.

CHARACTERISTIC FEATURES OF THE MICROBIOLOGY OF THE WEBSTER AND TAMA SILT LOAMS[1]

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Since the recognition of the existence of a microbiological population in the soil and the demonstration that the activities of the microorganisms concerned have a great influence on soil fertility, constant attempts have been made to construct some picture of the distribution of the soil population and to understand the relationships between the various groups of organisms composing it. Attempts to obtain an index of the microbiological condition of the soil by the determination of those biochemical changes that the population could bring about have in most cases been recognized as being artificial. The well-known plate and dilution methods have given interesting and valuable data on the types of organisms present and their individual potentialities but have added little to the study of the population as a whole. However, increasing success is following the more straightforward approach of direct examination, now that some of the many serious technical difficulties have been overcome.

This investigation was an attempt to follow by direct examination, supplemented by field microscopic observations, the characteristic features of the microbiology of two typical Iowa soils.

Both soils used in this study, a Tama silt loam and a Webster silt loam, are widely distributed upland types in their respective areas. The Tama silt loam, found in the Mississippi loess area, has a pH value of about 5.6 and the Webster silt loam, which is typical of the Wisconsin drift area, has a pH of about 6.6.

Since it is known that the addition of available organic matter to soils greatly influences microbial activity it was believed any fundamental microorganic differences between the two soils under investigation might be brought out or emphasized in their behavior on addition of green manure. In addition to the examination of the soils themselves, observations were therefore made on samples into which soybeans and alfalfa had been incorporated as green manure. Such work calls for methods which do not destroy the natural soil structure. Only two of the direct methods considered fulfill this requirement. One is the method of Kubiëna and the other the Rossi-Cholodny buried slide technique. A third method, the so-called "bodenstaub" technique proposed by Cholodny, and a modification of it, although involving destruction of the initial soil structure, was also used.

It was learned that during wet periods anaerobic decomposition of plant remains took place in the depressed areas of the Webster silt loam whereas in better drained areas the decomposition was aerobic. This was evidenced by the soggy condition of the cornstalks taken from below the soil surface in the former areas and the lower moisture content of those

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from the latter areas. Numerous mites and protozoa were seen in the pithy region of the soggy stalks.

Laboratory observations of the Kubiëna cultures revealed that well decomposed farmyard manure failed to cause any appreciable mycelial development in the Tama soil, whereas the addition of soybeans and alfalfa stimulated the development of an abundance of mycelium in both the Webster and Tama silt loams. An interesting feature of this increase was the succession of the fungi, which, though not identical in both soils nor in one soil with both additions, had distinct resemblances. Microscopic examination of plant residues taken from the Tama soil showed an abundance of actinomycetes. These microorganisms were also numerous in the Kubiëna cultures of this soil.

Apart from the succession of the fungi other interesting features brought out by the Kubiëna cultures were the differences between the soil forms and culture forms of Rhizopus, and the different fruiting habitats of Aspergillus and Rhizopus.

A noticeable difference between the two soils was revealed in the Kubiëna cultures which had received alfalfa and soybeans as additions. In each soil during the first few days there was a rapid development of fungi, possibly more in the Webster than in the Tama, this being followed by a decrease in abundance of new hyphae. The decrease was slow and extended over a considerable period of time in the Tama soil, while in the Webster soil there appeared to be an almost complete cessation of fungous development after the seventh to the twelfth day. The explanation for this difference may lie in the physical characteristics of the two soils.

The information acquired from the buried slides was practically the same as that from the Kubiëna cultures, except that the observation of bacteria was possible and their distribution could therefore be followed. Some groups of bacteria apparently were concerned more with the breakdown of previously elaborated fungous tissue than wth that of the plant additions.

The "bodenstaub" technique and its modifications failed to give any data comparable to either the Kubiëna cultures or the Rossi-Cholodny technique.

As far as the suitability of the various methods employed is concerned, that of Kubiëna seems to offer the greatest possibilities, particularly with respect to fungi. The results given by this technique were in accord with those obtained from the field investigations. The Rossi-Cholodny procedure is in reality a field method and it does provide related information supplementing that obtained from the Kubiëna cultures. It also gives a better picture of the bacteria than any of the other methods used in this study. The "bodenstaub" technique and its modification destroys the natural structure of the soil and therefore the conditions are so changed that the organisms developing may not truly represent the natural flora. The modification of using fresh soil particles containing plant residues is capable of giving much useful information, particularly with respect to the soil protozoa, the development of which is probably favored by the high moisture conditions.

The results of this work may be summarized as follows:

1. The Webster and Tama silt loams are alike in that in each the fungous activity was increased when green manure was added to the soil.

2. In each soil a definite succession of fungi followed the addition of green manure.

3. Most of the decomposition taking place in the Webster soil is normally anaerobic in wet periods except at the very surface.

4. Aspergillus and Rhizopus showed a difference in fruiting habitats.

5. The soil form of Rhizopus differed from the culture form with respect to the number of sporangiophores.

6. Certain groups of bacteria seemed to be almost entirely engaged in the decomposition of fungous tissues in the soil.

7. The numerous mites found in the pithy region of the cornstalks buried in the Webster silt loam indicated that these invertebrates may be important in the decomposition of plant residues.

8. Laboratory methods, such as the Kubiëna technique, which do not completely destroy the initial structure of the soil, more nearly simulate field conditions and yield information comparable to that obtained by field investigations.

9. The Rossi-Cholodny method offers a means of studying the influence of materials, such as green manure, added to the soil and at the same time preserving the natural habitats of the organism acting upon such materials.

10. The "bodenstaub" procedure and its modification have such limitations that their value as direct methods of soil investigation are questionable, although they may be used in protozoan studies.

FUNCTION OF VITAMIN B_1 IN ANAEROBIC BACTERIAL METABOLISM

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Since the relationship between the accumulation of pyruvic acid and the absence of vitamin B_1 in animal tissues has been pointed out by Peters and his associates, numerous studies have been undertaken to further clarify the function of vitamin B_1 in cellular metabolism and, as a result, investigation of the vitamin requirements of unicellular organisms has been greatly stimulated. Many important contributions have been made in papers too numerous to mention. The purpose of the present paper is to discuss the function of vitamin B_1 and its thiazole and pyrimidine fractions in the anaerobic pyruvate, lactate and glucose metabolism of the lactic and propionic acid bacteria.

THE CATALYTIC EFFECTS OF CRYSTALLINE VITAMIN B_1 ON THE ANAEROBIC PYRUVATE METABOLISM OF LACTOBACILLUS MANNITOPOEUS

Hills (1938) has reported a marked stimulation of the pyruvate metabolism of *Staphylococcus aureus* grown in vitamin B_1-deficient media by the addition of crystalline vitamin B_1 to cell suspensions. The present authors (1938) have shown that similar results are obtained in the case of the propionic acid bacteria, *Propionibacterium pentosaceum* and *P. peterssonii.* Data now presented show that the same catalytic effects of vitamin B_1 can be demonstrated for a member of the heterolactic bacteria, *Lactobacillus mannitopoeus.*

L. mannitopoeus grown in a yeast extract-peptone medium was transferred to the basal medium of Tatum, Wood and Peterson (1936) containing in addition 0.05 mg. of riboflavin and 7.5 mg. tryptophane per 100 gm. After incubation at 30° C. for 72 hours, the cells were washed twice in distilled water and suspended in M/15 phosphate buffer at pH 5.6. The cell suspension contained 1 gm. of cell paste in 40 cc. of buffer. One cc. of this cell suspension was tested for activity on sodium pyruvate both in the presence and absence of vitamin B_1. The results obtained on the Warburg respirometer are shown graphically in figure 1. The cells to which vitamin B_1 have been added show an increased rate of CO_2 evolution from pyruvate equal to four times that of the control.

L. mannitopoeus grown in the medium mentioned above containing in addition 10^{-8} gm. of vitamin B_1 per cc. shows no stimulation on the addition of crystalline vitamin to the cells. The rate of CO_2 evolution from pyruvate under anaerobic conditions remains unchanged (table 1).

VITAMIN B_1 CATALYSIS OF THE ANAEROBIC DISSIMILATION OF GLUCOSE AND SODIUM LACTATE BY P. PENTOSACEUM

It has already been shown that vitamin B_1 catalyzes the anaerobic dissimilation of pyruvic acid by *P. pentosaceum.* If pyruvic acid is an

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TABLE 1. *Anaerobic pyruvate dissimilation of L. mannitopoeus grown in presence of 10^{-8} gm. of vitamin B_1 per cc. of medium*

Treatment	Time, hours			
	0.5	1	1.5	2
	mm^3CO_2	mm^3CO_2	mm^3CO_2	mm^3CO_2
2 gamma B_1	25	54	89	124
No B_1 added to cell suspension	24	54	89	122

intermediate in the fermentation of glucose (Wood and Werkman, 1934), dissimilation of the latter by B_1-deficient *P. pentosaceum* should be stimulated by addition of the vitamin. The propionic acid bacteria were transferred from a yeast extract-peptone medium to the basal medium of Tatum, Wood and Peterson. The cells were collected at 72 hours by centrifugation, washed twice in distilled water and suspended in M/15 phosphate buffer pH 5.6 (one part by volume cell paste to 20 parts buffer). One cc. of this cell suspension was tested on glucose in the Warburg respirometer under an atmosphere of nitrogen at 30° C. All experiments with *P. pentosaceum* were run under these conditions.

Table 2 shows that the increase in the rate of breakdown of glucose due to added vitamin B_1, is not of the same order as that which occurs in the case of pyruvic acid. It is to be noted that as the concentration of pyruvic acid is decreased, the increase in its rate of dissimilation diminishes until at the lower concentrations it is of the same order as that of glucose. Assuming that the vitamin affects the dissimilation of glucose only after it has been broken down to pyruvate, and that the rate of dissimilation is a true index, we find under the conditions of our experiments that the amount of pyruvic acid arising from the glucose at any one instant is in the vicinity of 0.56 to 1.12 mg. However, after the first 2 hours the rate of dissimilation of glucose in the presence of vitamin B_1 rapidly approaches that in the absence of vitamin B_1.

Pyruvic acid is generally accepted to be an intermediate in the production of propionic acid from lactates by the propionic acid bacteria (van Niel, 1928; Erb, Wood and Werkman, 1936). In this case it is to be

TABLE 2. *Comparative effect of vitamin B_1 on the dissimilation of pyruvate and glucose by P. pentosaceum*

Substrate	Glucose		Pyruvate											
Mg. substrate per cup	13.0		9.0		4.5		2.25		1.12		0.56		0.28	
Vitamin B_1 gamma	0	1	0	1	0	1	0	1	0	1	0	1	0	1
CO_2/hr. mm^3	38	51	56	212	45	115	36	126	36	75	26	33	17	26

expected that vitamin B_1 will stimulate the formation of propionic acid from lactic acid. This stimulation is found to occur. The rates of anaerobic dissimilation of pyruvic and lactic acids are compared in table 3.

TABLE 3. *Comparative stimulation by vitamin B_1 in the dissimilation of pyruvate and lactate by P. pentosaceum*

Substrate	Lactate		Pyruvate	
Vitamin B_1 gamma	0	1	0	1
mm^3 CO_2/hr.	35	54	73	165
Per cent increase		54		126

The effect of the addition of crystalline vitamin to vitamin B_1 deficient cells on various substrates is shown in table 4.

Apparently vitamin B_1 acts as a stimulant in the dissimilation of all substrates tested with the exception of galactose. In this case no significant increase can be observed on the addition of the vitamin.

TABLE 4. *The dissimilation of several substrates in the presence and absence of vitamin B_1*

Treatment	Arabinose	Dihydroxyacetone	Glyceraldehyde	Mannitol	Galactose
No B_1	71	88	41	17	74
1 gamma B_1	192	211	106	47	85

Note: Substrate conc. 0.5 cc. of 0.8 per cent solution.
Results in mm^3 CO_2 evolved in 3 hours for arabinose and dihydroxyacetone, 4 hours for other substrates.

THE INFLUENCE OF THE PYRIMIDINE AND THIAZOLE FRACTIONS OF VITAMIN B_1 ON THE GROWTH AND METABOLISM OF P. PENTOSACEUM

Robbins (1938) has shown that certain fungi apparently synthesize vitamin B_1 in synthetic media, others may utilize either the pyrimidine or thiazole fraction and synthesize the other, while still other fungi can grow only in the presence of both fractions apparently being able to couple them. Using the same fractions employed by Robbins an experiment was undertaken to determine whether thiazole[2] or pyrimidine[3] alone or to-

TABLE 5. *Effect of vitamin fractions*

Activator	None	2 gamma thiazole	2 gamma pyrimidine	2 gamma thiazole 2 gamma pyrimidine	2 gamma vitamin B_1
mm^3 in 3 hrs.	141	143	128	141	271

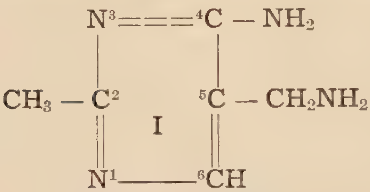
Substrate 9mg. Na pyruvate.

[2] Pyrimidine = 2-methyl-5-bromomethyl-6-aminopyrimidine hydrobromide.

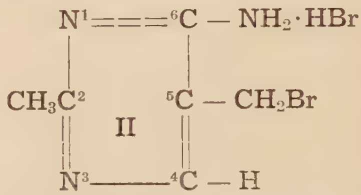
[3] Thiazole = 4-methyl-5-β-hydroxyethylthiazole.

gether can replace vitamin B_1. Table 5 shows that neither fraction alone, nor both together act as a stimulant in the pyruvate metabolism of *P. pentosaceum.*

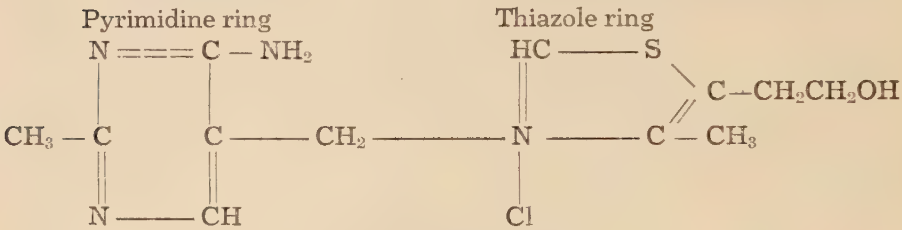
Apparently *P. pentosaceum* cannot couple the two fractions used to form vitamin B_1, unlike *Staph. aureus* whose pyruvate metabolism has been shown by Hills (1938) to be greatly stimulated in the presence of both pyrimidine and thiazole, but not by either alone. It may be that the difference is due to the slightly different pyrimidine fractions employed. Hills used 4-amino-5-aminomethyl-2-methyl pyrimidine while we employed the 2-methyl-5-bromomethyl-6-aminopyrimidine hydrobromide.



4-amino-5-aminomethyl-2-methyl pyrimidine (English nomenclature)



2-methyl-5-bromomethyl-6-amino-pyrimidine hydrobromide (American nomenclature)



Vitamin B_1 chloride

Growth experiments were undertaken to see whether growing cells of *P. pentosaceum* can utilize either or both the pyrimidine and thiazole fractions as a substitute for Vitamin B_1. The basal medium of Tatum, Wood and Peterson consisting of the acidic ether extract of yeast, and hydrolyzed casein with the addition of the two fractions and Vitamin B_1 was employed. The results are tabulated in table 6.

TABLE 6. *Non-stimulation of growing cells by vitamin fractions*

Flask	I	II	III	IV	V
Basal medium, cc.	150	150	150	150	150
Thiazole, gamma	.....	2	.....	2	.....
Pyrimidine, gamma	.....	.....	2	2	.....
Vitamin B_1, gamma	.....	.....	.....	.....	2
Yield of cell paste at 72 hours, cc.	0.29	0.32	0.34	0.28	0.44
Activity of grown cells on pyruvate, ie., CO_2 evolved in 2 hours	74	75	74	69	168

Apparently growing cells of *P. pentosaceum* cannot use either the pyrimidine fraction or thiazole fraction alone to replace the vitamin. Neither can the growing cells couple the two fractions to form the vitamin.

The explanation for the inability of *P. pentosaceum* to link the two fractions may lie in the location of the bromine atom in the 5-CH_2Br of the pyrimidine group. Knight and McIlwain (1938) have presented evidence indicating that linkage by *Staph. aureus* can occur when the substituted 5-CH_2- group is -CH_2NH_2, -CH_2OH or -$CH_2NH \cdot CSH$, but is not possible with -CH_2CONH_2 or -CH_3. Apparently *P. pentosaceum* cannot join the pyrimidine and thiazole fractions when the linkage must occur by means of 5-CH_2Br in the pyrimidine ring.

THE OCCURRENCE OF COCARBOXYLASE IN BACTERIAL CELL EXTRACTS

The authors (1939) have submitted evidence which indicates that vitamin B_1 is esterified to cocarboxylase by *P. pentosaceum.* We wish at the present time to point out the occurrence of cocarboxylase or the pyrophosphoric acid ester of vitamin B_1 in several types of bacteria.

One gram dried "bottom" brewer's yeast if washed rapidly 3 times in 50 cc. M/10 disodiumphosphate at 30° C. shows almost no carboxylase activity. Crystalline vitamin B_1 alone will not reactivate such yeast, whereas synthetic cocarboxylase and bacterial cell extracts do. In table 7 the comparative cocarboxylase contents of cell juices from *P. pentosaceum, Escherichia coli* and *Aerobacter indolgenes* are shown. The propionic acid bacteria were cultured in a yeast-extract, peptone, glucose medium; *E. coli* and *A. indologenes* were grown in a peptone, glucose, phosphate, tap-water medium. After 72 hours at 30° C., the cells were thrown out of suspension, washed twice in distilled water and dried in a vacuum desiccator at room temperature. The extracts were prepared by suspending one gram of the dried bacterial cells in 10 cc. of water, bringing the suspension to a boil and then centrifuging. The clear supernatant fluid was employed as cell extract.

The data leave little doubt that cocarboxylase occurs normally in the bacterial cells tested.

ACKNOWLEDGMENTS

We wish to express our thanks to Dr. Joseph Rosin of Merck and Company for supplies of pyrimidine and thiazole, and to Dr. R. T. Major, also of Merck and Company for a sample of synthetic cocarboxylase. To

TABLE 7. *Qualitative cocarboxylase assay of bacterial extracts*

Activator	None	Extract of P. pentosaceum	Extract of E. coli	Extract of A. indologenes	2 Gamma cocarboxylase
mm^3 CO_2 in 30 minutes	30	69	104	211	209

Substrate: 9 mg. sod. pyruvate + 1 mg. MgCl
Test cells: 100 mg. alkaline washed yeast in 1 cc. PO_4 M/15 pH 6.2
Extract: 0.5 cc.
Total vol.: 2.0 cc.
Atmosphere: air

Winthrop Chemical Company we are indebted for a generous portion of synthetic vitamin B_1. The "bottom" brewer's yeast was supplied through the courtesy of Anheuser-Busch, St. Louis, Missouri.

SUMMARY

1. Vitamin B_1 is essential for anaerobic dissimilation of pyruvic acid by the heterolactic acid bacterium, *Lactobacillus mannitopoeus*.
2. The anaerobic dissimilation of glucose, lactic acid, and several other substrates by *Propionibacterium pentosaceum* is stimulated by crystalline vitamin B_1.
3. 2-methyl-5-bromomethyl-4-aminopyrimidine hydrobromide and 4-methyl-5-β-hydroxyethylthiazole cannot replace vitamin B_1 either as a growth factor or metabolic stimulant in the enzyme system of *P. pentosaceum*.
4. Evidence is presented for the occurrence of cocarboxylase in bacterial cells.

REFERENCES

1. HILLS, G. M.
   1938. Aneurin (Vitamin B_1) and Pyruvate Metabolism by *Staphylococcus aureus*. Biochem. J. 32: 383-391.

2. SILVERMAN, M., and C. H. WERKMAN
   1938. Vitamin B_1 in Bacterial Metabolism. Proc. Soc. Exp. Biol. Med. 38: 823-827.

3. TATUM, E. L., H. G. WOOD, and W. H. PETERSON
   1936. Growth Factors for Bacteria. Vitamin B_1 a Growth Stimulant for Propionic Acid Bacteria. Biochem. J. 30: 1898-1904.

4. WOOD, H. G., and C. H. WERKMAN
   1934. Pyruvic acid in the Dissimilation of Glucose by the Propionic Acid Bacteria. Biochem. J. 28: 745-747.

5. VAN NIEL, C. B.
   1928. The Propionic Acid Bacteria. Haarlem.

6. ERB, C., H. G. WOOD, and C. H. WERKMAN
   1936. The Aerobic Dissimilation of Lactic Acid by the Propionic Acid Bacteria. J. Bact. 31: 595-602.

7. ROBBINS, W. J.
   1938. Organisms Requiring Vitamin B_1. Proc. Nat. Acad. of Sciences U.S.A. 24: 53-56.

8. KNIGHT, B. C. J. G., and H. MCILWAIN
   1938. The Specificity of Aneurin and Nicotinamide in the Growth of *Staph. aureus*. Biochem. J. 32: 1241-1251.

9. SILVERMAN, M., and C. H. WERKMAN
   1939. Bacterial Synthesis of Cocarboxylase. Enzymologia. 5: 385-387.

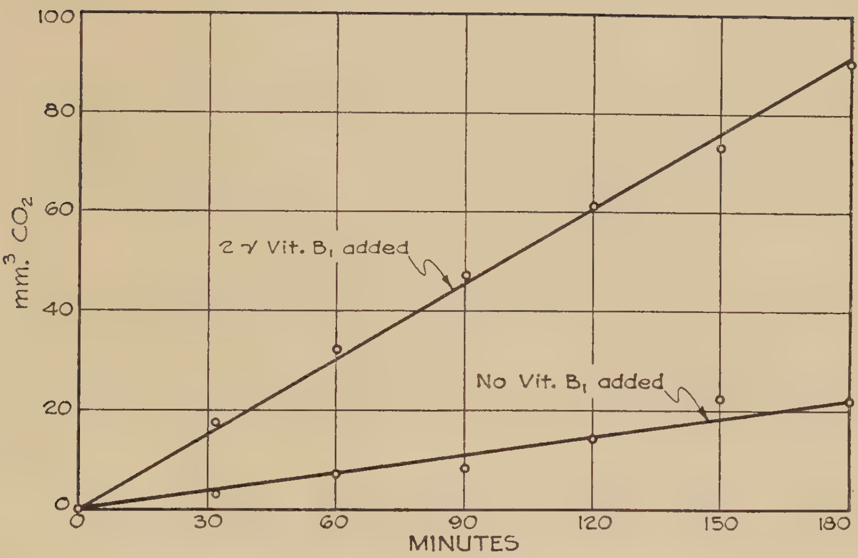


FIG. 1. The effect of vitamin B_1 on the anaerobic pyruvate metabolism of *Lactobacillus mannitopoeus.*

Substrate, 9 mg. pyruvic acid. Total vol., 2.0 cc. Atmosphere, nitrogen. Temp., 30° C.

THE DEHYDRATION OF CHOLESTEROL[1]

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The dehydration of cholesterol is an important phase in the investigation of the chemical antirachitic activation of sterols (11) since the activating reagents used are dehydrating reagents. However, the literature on the dehydration products of cholesterol is not only indefinite but also very confusing so that a correlation of the material in the literature seems desirable.

HISTORICAL

There are three general types of reactions by which the dehydration products of cholesterol are obtained: (a) direct dehydration of cholesterol, allocholesterol, or their epimers, (b) the removal of hydrogen halide from cholesteryl halides, and (c) the pyrolytic decomposition of cholesteryl esters. An attempt has been made to consult all references to the dehydration products of cholesterol. A list of these products is presented in table 1.

The variation in the reported melting points and optical rotations for the dehydration products of cholesterol has made difficult their characterization. The optical rotation of various cholesterol derivatives has been studied by Mauthner (27), Callow and Young (8) and Stavely and Bergmann (46); among other deductions it is interesting in this connection to note that those derivatives which have a double bond in the 4,5-position are dextrorotatory, whereas a double bond in the 5,6-position causes laevorotation.

Stavely and Bergmann (46) separated the cholesterilenes (cholesterilene prepared by some of the various methods) into two groups which have specific rotations (a) between —60° and —70° and (b) greater than —100° and suggested that both groups have the 3,5-conjugated system since a member of each group possessed a similar absorption spectrum. However, the same authors (46, 47) used the terms cholesterilene and 3,5-cholestadiene interchangeably and also suggested (47) that cholesterilene prepared by the zinc dust distillation of cholesterol (14) was a mixture of cholesterilene and 2,4-cholestadiene.

Cholesterilene was previously assigned the structure of 2,5-cholestadiene by Heilbron (20) but the high absorption of cholesterilene in the ultraviolet is considered indicative of a conjugated system (46). Three maxima reported at 249, 304, and 312 mμ in the absorption spectrum of cholesterilene (21) were later not confirmed (37, 43) but instead other maxima at 229, 235 and 244 mμ (44, 46) and at 235 and 245 mμ (23) have been found. Other physical constants of cholesterilene such as crystal structure and refractive index (1, 46, 52) have been reported.

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[1] Journal Paper No. J-599 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 506.

[2] Part of the literature search was conducted by Ralph L. Van Peursem in partial fulfilment for the degree of Doctor of Philosophy.

Heilbron and Sexton (22) suggested that one double bond in cholesterilene must occupy the same position as the double bond of pseudocholestene since the formation of pseudocholestene by the action of heat on cholesterol was regarded as a result of dehydrogenation of part of the cholesterol to cholestenone and then the hydrogen liberated was used to reduce cholesterilene, obtained by dehydration of a further part of the cholesterol, to pseudocholestene. Windaus (58) concluded that cholesterilene did not possess a conjugated system of double bonds since is was recovered unchanged after treatment with sodium in boiling absolute alcohol. Sodium in boiling amyl alcohol has also been found (14, 46, 58) not to affect cholesterilene.

Although cholesterilene must possess two double bonds since it absorbs four atoms of hydrogen, Windaus (59) suggested that cholesterilene may consist of several isomers which only slightly influence each other's melting points and form mixed crystals with each other and also that the variation in optical rotation of cholesterilene, prepared by various methods, can not result from a partial racermization since the products—cholestane and coprostane—obtained by catalytic hydrogenation possessed the proper optical rotations. Cholestane or coprostane or both have been isolated from the catalytic reduction products of cholesterilene in ether, ethyl acetate or acetone solution with platinum or palladium catalyst (7, 14, 38, 46, 49, 50, 59, 61). Cholesterilene was also found to be reduced to cholestane by the action of selenium (10).

Chlorine has been reported (64) to decompose a-cholesterilene (moleuclar weight 482 (28)), b-cholesterilene and c-cholesterilene (molecular weight 764, 780, 769 and 721 (28)) and to evolve hydrogen chloride (65) from a- and b-cholesteron. Iodine values of 70.9, 70.0, 72.2 (28), 73.36 and 77.87 (37) have been recorded for cholesterilene; this corresponds to approximately one mole of iodine per mole of cholesterilene.

Mauthner and Suida (28) believed that cholesterilene possessed only one double bond since it added only one mole of bromine. However, Windaus (58) considered it likely that cholesterilene contained two double bonds which absorbed bromine by 1,4-addition and created a double bond which was inactive to the addition of more bromine. Cholesterilene dissolved in chloroform, carbon disulfide or acetic acid has been found to add one mole of bromine (12, 29, 32, 46, 50, 51) without the isolation of a bromine addition product (17, 29, 46) and that additional bromine was not immediately decolorized but hydrogen bromide was evolved (29) to yield a brown liquid (46). Attempts to recover cholesterilene, after treatment with bromine, by the action of sodium iodide yielded black tarry material (46). Mention has been made (26) of a tetrabromocholestane, obtained by the action of bromine on cholesterilene, which was not described except that it was different from tetrabromocholestane, m.p. 110°, obtained by the action of bromine on *epi*cholesteryl acetate; the tetrabromocholestane from cholesterilene was not confirmed (46).

Walitsky (56) stated that c-cholesterilene of Zwenger (64) and "cholestene" obtained by the action of hydrogen iodide or of sodium or cholesterol all yielded the same bromine compound $C_{26}H_{34}Br_8$ with the evolution of hydrogen bromide when treated with an excess of bromine. The product $C_{27}H_{44}$, m.p. 78-9°, obtained by the action of sodium ethoxide on "4-dichlorocholestene" was reported to give a tetrabromide melting at 162-4° (41). It has also been reported that from the mother liquors, after the removal of cholesterilene form the reaction product obtained by the

action of phosphorus pentoxide on cholesterol (33), was obtained a compound, m.p. 66-7°, which with bromine in chloroform solution gave a bromide melting at 61-2°.

A maleic anhydride addition product was not obtained by treating cholesterilene with maleic anhydride in benzene (46) or toluene (53) solution although an abnormal addition product, m.p. 240-5° with decomposition, was obtained in xylene solution heated in a sealed tube (46). If cholesterilene contains the 3,5-conjugated system, addition would have to take place in an unusual manner due to sterochemical reasons (46).

When treated wtih perbenzoic acid, cholesterilene has been found to require 1.39 and 1.49 moles of perbenzoic acid per mole of cholesterilene after 24 hours, 1.56 and 1.66 moles after 48 hours and 1.63 and 1.74 moles after 82 hours (44). Perbenzoic acid titration of cholesterilene has also been observed to indicate 1.97 double bonds after 48 hours (46) and 1.87 double bonds after 8 days (12).

Cholesterilene was found to be oxidized by the action of posassium permanganate (29, 51) and an acid containing 27 carbon atoms was isolated by chromic acid oxidation (29). Oxidation of cholesterilene with chromium trioxide yielded cholestene-4-dione-3,6 (14, 58) which was found to be identical with the neutral oxidation product from 3,5-cholestadiene (isolated as the monophenylhydrazone) (12).

The unsaturated nature of cholesterilene was demonstrated by the tetranitromethane color reaction (50). Cholesterilene has been observed to give the usual cholesterol color reactions (6, 36, 44, 50, 51, 57). Whitby (57) suggested that, in the typical cholesterol reactions, cholesterilene was first formed and that it then coupled with a second substance, such as formaldehyde, to give the colored products. However, Wokes (62) was not able to obtain any colors by the treatment of cholesterilene with formaldehyde alone (either as formalin solution or in the nascent condition).

Cholesterilene treated with sulfuric acid has been observed to give a brownish yellow solution with a strong green fluorescence (28), a citron yellow color (6) or a yellow solution with a green fluorescence (32). Soft brownish red resinous masses were obtained by the action of sulfuric acid on a-, b- and c-cholesterilene (64). Nitric acid was reported to slightly attack a-, b- and c-cholesterilene (64), with the formation of Redtenbacher's acid (42) from a-cholesterilene. Cholesterilene treated with nitric acid was found to give a cherry red color (32) or a slight rose to cherry red color which was destroyed to a yellow resin by the addition of sodium nitrate (28). An ether or ether-alcohol solution of cholesterilene treated with hydrogen chloride displayed a color change through yellow to light brown to brownish red with a green fluorescence and the product obtained was found to be antirachitic (11b, 11c). Cholesterilene can also be converted into antirachitic products by other dehydrating acidic reagents but not by ultraviolet irradiation (11b, 11c, 11e). Treatment with a cold mixture of sulfuric acid and acetic anhydride was found to convert cholesterilene into cholesterilene sulfonic acid (methyl ester, m.p. 175-6°; lithium salt, turned brown at 220°) which was antirachitically inactive (60).

Treatment with alcoholic sulfuric acid was reported to convert cholesterilene in cholesterol which was identified by its melting point, optical rotation and bromine derivative (33). It was likewise found that treatment with dilute sulfuric acid in benzene-acetic acid solution converted cholesterilene into cholesterol which was identified as the

digitonide (14) although a chloroform solution of cholesterilene remained unchanged when shaken with 10 per cent sulfuric acid for 6 days (58).

The normal ether of cholesterol, dicholesteryl ether, formed by the removal of one molecule of water from two molecules of cholesterol has sometimes been called cholesteryl ether, cholesteryl oxide, β-cholesterylene or tricholesterol. It has been prepared by various methods (2, 3, 5, 17, 18, 28, 32, 34, 35, 36, 48, 49), forms a tetrabromide which melts at 164-6° (28, 32) (m.p. 178° (18)), gives sterol color reactions (3, 62), shows no marked selective absorption (21), is converted into an antirachitic product by treatment with sulfuric acid-acetic anhydride (11e) but is not activated by ultra-violet irradiation (3, 4, 19), reacts with hydrogen peroxide in benzene solution followed by acetylation to yield a diacetate melting at 143° (40), and when distilled with aluminum chloride gives various hydrocarbon fractions (63).

2,4-Cholestadiene has been prepared by the action of aluminum oxide on cholesterol (45, 47) and has been found to be reduced catalytically to coprostane, to react with perbenzoic acid in an amount equivalent to 1.94 double bonds, to give a normal maleic anhydride addition product melting at 70-2°, to be reduced to pseudocholestene by the action of sodium in alcohol, to be rearranged to cholesterilene by the action of alcoholic hydrochloric acid, to have a maxima in its absorption spectrum at 260 mμ (47) and to form a stable crystalline peroxide, m.p. 118.5-20.5°, $(\alpha)^{23}_{D} + 52.8$ in chloroform, by the action of oxygen in the presence of eosin (45).

EXPERIMENTAL

Preparation of a-cholesterilene. The a-cholesterilene of Zwenger (64) was prepared as follows. In a 500 cc. Erlenmeyer flask were placed 10 gm. of dry cholesterol and 40 cc. of dilute sulfuric acid (1 volume of concentrated sulfuric acid diluted with 1 volume of water). The reaction mixture was immersed in a water bath maintained at 65° and 60 cc. of concentrated sulfuric acid were added with stirring in the period of 1 minute, during which time the colorless suspension changed through pink to a dark red mass. The reaction mixture was heated an additional 2 minutes at 65° (bath temperature) with stirring and the slightly fluorescent sulfuric acid was immediately removed by decantation. The residue was washed twice with water by decantation and then allowed to slowly hydrolyze in 50 cc. of water; on treatment with water the dark red mass slowly changed through a purple color to a pale yellow suspension. The hydrolysis was aided by smashing the lumps with a stirring rod. The water suspension was made slightly alkaline, to remove all the sulfuric acid, by the addition of 10 cc. of ammonium hydroxide and then 60 cc. of alcohol were added to increase the difference between the density of the suspending medium and that of the suspensoid. The suspension was centrifuged and the pale yellow solid was centrifuged several times from suspension in 50 per cent alcohol and from 95 per cent alcohol. One hundred cubic centimeters of ether was added to the air dried product (7.5 gm.) and the ether-insoluble a-cholesterilene was removed by centrifugation and then centrifuged from ether suspension three times. The a-cholesterilene, yield 1 gm., was recrystallized from chloroform solution until it melted at 344° (bloc Maquenne), $(\alpha)^{23}_{D} + 96.85$ (c, .8096 in carbon tetrachloride). The melting point of a-cholesterilene was reported by Zwenger (64) to be 240° but Mauthner and Suida (28) found it to sinter at 210-20°, to become trans-

parent at 235° and to become fluid at 260°. a-Cholesterilene was found to display the capillary melting point of 240-65° with decomposition so the bloc Maquenne melting point was considered more reliable for melting point and mixed melting point determinations.

Preparation of c-cholesterilene. The ether solutions containing the ether-soluble fraction of the reaction product were combined and concentrated *invacuo.* The residue was dissolved in benzene-petroleum ether (1:1 by volume) and the solution was passed through a column of activated alumina. The solution was concentrated *in vacuo* and the residue was recrystallized from ether-alcohol until it melted at 200° (bloc Maquenne), $(\alpha)^{23}_{D} + 34.5$ (c, 2 in carbon tetrachloride). The yield of this compound, the c-cholesterilene of Zwenger, was 3.5 gm. The melting point of c-cholesterilene was reported by Zwenger (64) to be 127° but Mauthner and Suida (28) did not record a melting point. Similar to a-cholesterilene, c-cholesterilene was also found to display a decomposing capillary melting point (m.p. 144-72° with decomposition). A compound having the characteristics of the b-cholesterilene of Zwenger (64) was not isolated from the reaction product obtained by the action of sulfuric acid on cholesterol.

Bromination of a- and c-cholesterilenes. A slight excess of bromine in chloroform solution was added to a chloroform solution of a-cholesterilene and it was observed that the bromine was slowly absorbed with the evolution of hydrogen bromide. The solvent was removed *in vacuo* and the residue was recrystallized from ether-alcohol until it melted at 245° (bloc Maquenne) (capillary melting point 135-47° with decomposition).

An excess of bromine in acetic acid solution was added to an ether solution of c-cholesterilene and it was likewise observed that the bromine was absorbed with the evolution of hydrogen bromide. The solution was poured into water and extracted with ether. The ether solution was washed with water and dried over anhydrous sodium sulfate. Alcohol was added to the dried ether solution and the product obtained was recrystallized from ether-alcohol until it melted at 235° (bloc Maquenne) (capillary melting point 163-78° with decomposition).

Treatment of cholesterol with other reagents. a-Cholesterilene was isolated from the reaction products obtained by the action of a number of reagents on cholesterol and some cholesterol derivatives. Various methods of preparing a-cholesterilene are presented in a condensed form in table 2 and the antirachitic activity of the ether soluble fractions of the reaction products obtained by these methods is presented elsewhere (11a, 11c, 11d).

DISCUSSION

A study of the dehydration of cholesterol leads to the obvious conclusion that a number of products can be prepared. The compound or compounds obtained depends on the procedure employed. Thus one molecule of water can be removed from one molecule of cholesterol with the formation of 2,4-cholestadiene or of cholesterilene which is considered identical with 3,5-cholestadiene although the variation in the optical rotation of cholesterilene prepared by various methods has not been explained. Under other conditions, one molecule of water can also be removed from two molecules of cholesterol with the formation of dicholesteryl ether. The formation of 3,5-cholestadiene and of dicholesteryl ether requires only dehydration but the formation of 2,4-cholestadiene requires a shift of the

TABLE 1. *Dehydration products of cholesterol*[1]

Compound (name used in reference)	Method of preparation	M. P. in °C	Optical rotation $(\alpha)_D$	Optical rotation °C	Optical rotation c or (p)	Optical rotation Solvent	Actually or (suggested) identical with in reference	Reference
a Cholesterilin	cholesterol + H_2SO_4	240						64
a–Cholesterilin	" or cholesterilene + H_2SO_4	210–60						28
b Cholesterilin	cholesterol + H_2SO_4	255						64
c Cholesterilin	" + "	127						64
c–Cholesterilin	" + "	68–100						56
Cholesteryl ether	" + "	74.5						31
" "	" + "	195–201						31
Substance	" + "	135–6					(cholesterol?)	31
Cholesterylenes	" + "	188–93						35
β–Cholesterylene	" + H_2TeO_4	68					cholesteryl oxide	35
a Cholesteron	" + H_3PO_4	79.5						65
a–Cholesteron	" + "	80.5					(cholesterilen)	28
b Cholesteron	" + "	175						65
b–Cholesteron	" + "	192					(cholesteryl ether)	28
α–Cholesterylene	" + H_3PO_4 in HOAc	79–80						32
β–Cholesterylene	" + $KHSO_4$	188–93					cholesteryl oxide	32
Powder	" + HI	68					hydrocarbon $C_{26}H_{42}$	54
Cholestene	" + "	68–100						56
Powder	" + " + P_4	68					hydrocarbon $C_{26}H_{42}$	54
Hydrocarbon $C_{26}H_{44}$	" + " + "	68						24
Hydrocarbon $C_{26}H_{42}$	" + Na	68–100					cholestene (cholesterilene)	56
Benzyl cholesteryl ether	Na cholesteroloxide + $C_6H_5CH_2Cl$	78						39
" " "	Na cholesteroloxide + $C_6H_5CH_2Cl$	80–4					cholesterylen	48
Cholesteryl ether	Na cholesteroloxide + EtI	141						25
" "	Na cholesteroloxide + EtI						not cholesteryl ether	28
α–Cholesterylene	cholesterol + $HClO_4$	78–80						34
β–Cholesterylene	" + "	193					cholesteryl oxide	34
Cholesteryl ether	" + HCl	74.5						31
α–Cholesterylen	cholesterol + HCl gas							17
Cholesterilene	epicholesterol + HCl	76–7	— 78.3	30	(1)	C_6H_6		26
Hydrocarbon	epiallocholesterol + HCl	79	—112.8	23	(2)	"	(cholesterilene)	13

(Continued on next page)

TABLE 1. *(continued)*

Compound (name used in reference)	Method of preparation	M. P. in °C	Optical rotation $(\alpha)_D$	°C	c or (p)	Solvent	Actually or (suggested) identical with in reference	Reference
2,4-Cholestadiene	allocholesterol + HCl	79	—112.5	23				44
"	epiallocholesterol + HCl	79						44
"	allocholesterol[2] + HCl	79	—108.2	24				44
"	allo- or epiallocholesterol + HCl						cholesterilene	46
Cholesterilene	allo- or epiallocholesterol + HCl	79.5-80	—123.23	25	3.00	CCl_4	3,5-cholestadiene	12
2,4-Cholestadiene	cholesterol + Al_2O_3	63	+114.0	27	1.523	$CHCl_3$		47
"	" + "	68.5	+168.5			ether		45
Cholesterilene	" + "	79.5-80	— 51.4	20		"		45
α-Cholesterylen	" + P_2O_5	79-80						33
Cholesterilen	" + $CuSO_4$	79-80						28
"	" + "	79-80	— 81.63					30
Cholesterilene	" + "	74	— 71.88	18	0.626	C_6H_6		6
"	" + "	78	—102.1	20	4.000	$CHCl_3$		21
"	" + "	78-9	— 97.5	20	0.9	"	(3,5-cholestadiene)	46
"	" + "	79.5-80	—104.91	25	3.00	CCl_4	3,5-cholestadiene	12
Cholesterilene	" + CuCl or AgCl	78						16
"	cholesterol + CuS							15
"	" + kiesselguhr	79	— 46.6		4.504	$C_6H_4CH_3$		49
"	" + fossil flour	78-9						15
Cholesterilen	" + zinc dust[3]	68	+ 1.45	26	2.75			14
		73	— 53.37	22	2.08			
		75	— 4.49	20	2.34			
α-Cholesterylene	" + HgI_2	79-80						36
"	cholesteryl chloride distilled	79	— 47.04					49
Cholesterilen	cholesteryl chloride + CaO	79	— 61.55					30
"	cholesteryl chloride + quinoline	77	— 86.09					30
Cholesterilene	cholesteryl chloride + quinoline	79.5-80	—100.33	25	3.00	CCl_4	3,5-cholestadiene	12
Cholesterylen	cholesteryl chloride + K cholesteroloxide	79-80						48
Cholesteryl ether	cholsteryl-chloride + Na cholesteroloxide	71						25
" "	cholsteryl-chloride + Na cholesteroloxide						not cholesteryl ether	28

(Continued on next page)

TABLE 1. *(continued)*

Compound (name used in reference)	Method of preparation	M. P. in °C	Optical rotation $(\alpha)_D$	°C	c or (p)	Solvent	Actually or (suggested) identical with in reference	Reference
Cholesterylen	cholsteryl chloride + ZnO	79–80	—116.2		2.641	$CHCl_3$		48
"	cholesteryl chloride + Zn							48
Cholesterilene	cholesteryl chloride + NH_3	79–80						41
Cholesterilene (several)	cholesteryl chloride + NH_3	82 to 256						9
Hydrocarbon $C_{28}H_{42}$	cholesteryl chloride + NaOEt	80						54
Cholesterilen	cholesteryl chloride + NaOEt	79–80						28
Cholesterilen	cholesteryl chloride + NaOEt	80	— 65.7					55
Product $C_{27}H_{44}$	4-dichlorocholestene + NaOEt	78–9						41
Cholestadien	cholesteryl bromide + NaI	77–8	— 65.4	19	0.541	$C_6H_4CH_3$		53
"	cholesteryl bromide + NaI + piperdine acetate	77–8	—103					53
α-Cholesterylene	cholesteryl methyl xanthogenate	77	—107		(11)	"		51
"	cholesteryl methyl xanthogenate	77	—109.3	20	(4.503)	"		50
"	cholesteryl methyl xanthogenate	79–80						7
β-Cholesterylene	cholesteryl methyl xanthogenate	59	— 76.08	20	(3.785)	"	cholesterilene	50
"	cholesteryl methyl xanthogenate	79–80					"	7
Cholesterilene	cholesteryl methyl xanthogenate	79.5–80	—123.23	25	3.00	CCl_4	3,5-cholestadiene	12
"	cholesteryl phenyl urethane	75.5	—100.25		1.04	C_6H_6		6
α-Cholesterilen	cholesteryl oleate	79						17
Cholesterilene	monocholesteryl phosphoric acid	76–8	— 68.99	20				37
Cholesterilene	dicholesteryl phosphoric acid	78.2	— 77.53	18				37
Cholestadiene	4-cholestene oxide + alc HCl	81	— 71.7					23
"	α- 5-cholestene oxide + alc. HCl	80–1	— 68.5					23
"	5-hydroxy-4-acetoxycholestane + alc. HCl	80–1	— 68.3					23
"	5-hydroxy-6-acetoxycholestane + alc. HCl	80	— 68.7					23
Tricholesterol	cholesterol + floridin	203–9	— 40.8	20	(2)	CCl_4		3
3,5-Cholestadiene	7-ketocholesterilene semi-carbazone + NaOEt	78–9	— 63.75	21	1.026	$CHCl_3$	cholesterilene	46
"	4,5-dibromocholestane + quinoline	79.5–80	—103.23	25	3.00	CCl_4	"	12

[1] Not complete in the case of dicholesteryl ether
[2] Prepared from cholesterol hydrochloride
[3] Constants observed during purification

TABLE 2. *Various methods of preparation of a-cholesterilene*

Compound treated		Reagent used		Solvent used				
Compound	Amount in moles	Reagent	Amount in moles	Solvent	Amount in cc.	Temp. in °C	Time in hrs.	Yield in gm.
Cholesterol	0.001	H_2SO_4	0.002	HOAc	4	85–90	3	0.0515
Cholesterilene	0.001	"	0.002	"	4	85–90	3	0.0425
Cholesteryl acetate	0.001	"	0.002	"	4	85–90	3	0.0313
Butyl cholesteryl ether	0.001	"	0.002	"	4	85–90	3	0.0335
Cholesteryl chloride	0.001	"	0.002	"	4	85–90	3	0
Cholestene	0.001	"	0.002	"	4	85–90	3	0
Dicholesteryl ether	0.0005	"	0.004	"	8	85–90	3	0*
Cholesterol	0.001	HO_3SCH_2COOH	0.002	"	4	85–90	3	0.0385
"	0.001	H_2SO_4 + Ac_2O	0.002 0.0025	"	4	25	192	0.0145
"	0.001	H_2SO_4 + Ac_2O	0.002 0.0025	"	4	85–90	3	0
i-Cholesterol	0.001	H_2SO_4 + Ac_2O	0.002 0.0025	"	4	85–90	3	0.0135
Cholesterol	0.004	$ZnCl_2$	0.0146	"	16	85–90	3	0.437
"	0.00259	"	0.022	——	—	225	0.25	0
"	0.002	$ZnCl_2$ + Ac_2O	0.0073 0.005	HOAc	8	85–90	3	0.140
"	0.002	$ZnCl_2$ + AcCl	0.0073 0.005	"	8	85–90	3	0.082
Cholesterilene	0.002	$ZnCl_2$ + AcCl	0.0073 0.005	"	8	85–90	3	0.147
Cholestene	0.004	$ZnCl_2$ + AcCl	0.0146 0.01	"	16	85–90	3	0
Cholesterol	0.005	P_2O_5	0.007	C_6H_6	40	80	2	0.0785
Cholesterilene	0.005	"	0.007	"	40	80	2	0.1095
Cholestene	0.005	"	0.007	"	40	80	2	0
Cholesterilene	0.0068	CCl_3COOH	0.0482	——	—	25	6	0.0438
"	0.00262	"	0.0193	——	—	160	0.16	0

* Part of the dicholesteryl ether was recovered unchanged.

5,6-double bond to the 4-5-position followed by dehydration to form the 2,3-double bond.

The formation and characterization of other dehydration products, such as the a- and c-cholesterilenes of Zwenger, have been neglected. The directions for the preparation of these two compounds were studied and the formation of a-cholesterilene by a number of methods was found. Due to the differences in melting points, optical rotations and bromination products, these two compounds, a- and c-cholesterilenes, cannot be identical with 2,4-cholestadiene, cholesterilene or dicholesteryl ether.

Since a-cholesterilene was found to be formed from either cholesterol or cholesterilene, it is evident that dehydration is involved in its formation from cholesterol. It was also found that a-cholesterilene could be obtained from cholesteryl acetate, butyl cholesteryl ether or i-cholesterol but not from cholestene, cholesteryl chloride or dicholesteryl ether. Similar to the action of sulfuric acid, sulfoacetic acid also caused the formation of a-cholesterilene. It was isolated from the reaction product obtained by the action of sulfuric acid-acetic anhydride on cholesterol at 25° but not when the reaction mixture was heated at 85-90° at which temperature it was formed from i-cholesterol. a-Cholesterilene was produced by the action of zinc chloride on cholesterol in acetic acid solution but not when heated in the absence of a solvent. It was formed in the reaction mixtures obtained by the action of zinc chloride-acetyl chloride in acetic acid solution or of phosphorus pentoxide in benzene solution with cholesterol and cholesterilene but not from the reaction mixtures with cholestene. a-Cholesterilene was isolated from the reaction product obtained by the action of trichloracetic acid on cholesterilene at 25° but not when heated at 160°.

Since heat is not required in the formation of a-cholesterilene, it cannot be considered a product of pyrolysis. The observation that a-cholesterilene can be obtained by the action of trichloroacetic acid on cholesterilene at room temperature suggests that it is a rearrangement product since trichloroacetic acid may act as an isomerizing reagent. Furthermore, since a-cholesterilene can be obtained by the action of sulfuric acid-acetic anhydride at 85-90° from i-cholesterol but not from cholesterol under the same conditions and since i-cholesterol, a-cholesterilene and c-cholesterilene are all dextrorotator, it is possible that a- and c-cholesterilene may have some connection with i-cholesterol.

The variation in the optical rotation of cholesterilene prepared by various methods has not been explained. It can be seen that if dehydrogenation, hydrogenation or a rearrangement should occur along with dehydration to form a dextrorotatory by-product, such as cholestenone, pseudocholestene, a- or c-cholesterilenes, in the preparation of cholesterilene, then the optical rotation of the cholesterilene would depend on the removal of such impurities from the cholesterilene. The investigation is being continued.

SUMMARY

The literature on the dehydration of cholesterol including the preparation of the dehydration products with their physical and chemical properties has been reviewed. Five products—2,4-cholestadiene, cholesterilene, dicholesteryl ether, a-cholesterilene and c-cholesterilene—should be recognized as different dehydration products which can be obtained from cholesterol.

a- and c-Cholesterilenes were prepared by various methods and were found to be dextrorotatory and to yield bromination products. The formation of dextrorotatory dehydration products in the preparation of cholesterilene which as impurities would lower the negative optical rotation of cholesterilene was suggested as a possible explanation for the variation in the reported optical rotation of cholesterilene obtained by various methods.

BIBLIOGRAPHY

Note: An attempt has been made to include all references to the dehydration of cholesterol.

1. Auwers, K. v., and E. Wolter
1931. Nachr. Ges. Wiss. Gottingen Math.-physik. Klasse, **1931**:101-119; Chem. Abst., **26**:5844.

2. Beynon, J. H., I. M. Heilbron, and F. S. Spring
1936. Sterol group. XXV. Investigation of some reactions of the isomeric ethers of cholesterol. J. Chem. Soc., **1936**:907-910.

3. Bills, C. E., and F. G. McDonald
1926. Antiricketic substances. IV. The polymerization of cholesterol. J. Biol. Chem., **68**:821-831.

4. Bills C. E., and F. G. McDonald
1927. The catalytic formation of mixed cholesterol ethers. J. Biol. Chem. **72**:1-11.

5. Bills, C. E., and F. G. McDonald
1927. Antiricketic substances. V. The action of ultra-violet rays on the ethers and esters of cholesterol. J. Biol. Chem., **72**:13-19.

6. Bloch, A.
1904. Action de l'isocyanate de phenyle sur quelques alcools univalents (II). Bull. soc. chim., **31**:71-76.

7. Bose, A. C., and W. Doran
1929. Studies in the sterol group. Part V. The constitution of cholesterilene. J. Chem. Soc., **1929**:2244-2248.

8. Callow, R. K., and F. G. Young
1936. Relations between optical rotatory power and constitution in the steroids. Proc. Roy. Soc. (London), **157A**:194-212.

9. Diels, O., and E. Abderhalden
1904. Zur Kenntniss des Cholesterins. Ber., **37**:3092-3103.

10. Doree, C., and V. A. Petrow
1934. A case of hydrogenation in the sterol group by the action of selenium. J. Chem. Soc., **1934**:1129-1130.

11a. Eck, J. C., and B. H. Thomas
1937. The chemical activation of sterols. III. The chemical activation of cholesterol. J. Biol. Chem., **119**:621-630.

b. Eck, J. C., and B. H. Thomas
1937. The chemical activation of sterols. IV. The chemical activation of cholesterol and cholesterilene by various reagents. J. Biol. Chem., **119**:631-640.

c. Eck, J. C., and B. H. Thomas
1939. The chemical activation of sterols. VI. A study of various reagents in chemical activation and sterol color reactions. J. Biol Chem., **128**:267-278.

d. Eck, J. C., and B. H. Thomas
1939. The chemical activation of sterols. V. A study of the rlationship between

chemical activation and configuration of various sterols and derivatives. J. Biol. Chem., 128: 257-265.

e. Eck, J. C., B. H. Thomas, and L. Yoder
1937. The chemical activation of sterols. II. The chemical activation of cholesterol and various cholesterol derivatives. J. Biol. Chem., 117: 655-661.

12. Eck, J. C., R. L. Van Peursem, and E. W. Hollingsworth
1939. The preparation of 3,5- and 4,6-cholestadienes and a study of cholesterilene and of "7-dehydrorcholestene isomer." J. Am. Chem. Soc., 61: 171-174.

13. Evans, E. A., Jr., and R. Schoenheimer
1936. Epi-allocholesterol, a new isomer of cholesterol. J. Am. Chem. Soc., 58: 182.

14. Fantl, P.
1926. Zinkstaubdestillation des Cholesterins. Monatsh., 47: 251-258.

15. Fazi, R. de
1931. Sulla constituzione chimica della colesterina.—Nota I. Gazz. chim. ital., 61: 369-373.

16. Fazi, R. de, F. Monforte, and F. Pirrone
1932. Sulla cosituzione chimica della colesterina.—Nota VII. Azione del cloruro di argento e del cloruro remeoso sulla colesterina. Gazz. chim. ital., 62, 108-118.

17. Fischer, H., and A. Treibs
1926. Beitrag zur Kenntnis des Cholesterins. Ann., 446, 241-259.

18. Hattori, K., and K. Kawasaki
1936. Action acid clay on sterols. I. Colorimetric reaction or ergosterol and allocholesterol. J. Pharm. Soc. Japan, 56: 697-702.

19. Hess, A. F., and A. Windaus
1926. Experiments on activation of cholesterol derivatives and allied sterols by ultraviolet irradiations. Proc. Soc. Exptl. Biol. Med., 24: 171-172.

20. Heilbron, I. M.
1934. Dictionary of Organic Compounds. Oxford University Press, New York City, Vol. 1, p. 337.

21. Heilbron, I. M., R. A. Morton, and W. A. Sexton
Studies in the sterol group. Part I. The absorption spectra of some cholesterol derivatives. J. Chem. Soc., 1928: 47-51.

22. Heilbron, I. M., and W. A. Sexton
Studies in the sterol group. Part II. The formation of ψ-cholestene and cholestenone by the dry distillation of cholesterol. J. Chem. Soc., 1928: 347-351.

23. Heilbron, I. M., W. Shaw, and F. S. Spring
1938. Preparation and reactions of mono- and dihydroxy cholestanes. Rec. trav. chim., 57: 529-534.

24. Liebermann, C.
1885. Ueber das Oxychinoterpen. *Ber.*, 18: 1803-1809.

25. Lindenmeyer, O.
1863. Beitrage zur Kenntniss des Cholesterins. J. prakt. Chem., (1) 90: 321-331.

26. Marker, R. E., O. Kamm, T. S. Oakwood, and J. F. Laucius
1936. Sterols. VII. Cis and trans 3-carboxyandrostanone, an oestrus-producing male hormone derivative, and epi-cholesterol. J. Am. Chem. Soc., 58: 1948-1950.

27. Mauthner, J.
1906. Neue Beitrage zur Kenntnis des Cholesterins. II. Uber das Drehungsvermogen einiger Cholesten- und Cholestankorper, Monatsh., 27: 421-431.

28. MAUTHNER, J., and W. SUIDA
1896. Beitrage zur Kenntniss des Cholesterins. (III. Abhandlung) Monatsh., **17**:29-49.

29. MAUTHNER, J., and W. SUIDA
1896. Beitrage zur Kenntniss des Cholesterins. (IV. Abhandlung) Monatsh., **17**:597-603.

30. MAUTHNER, J., and W. SUIDA
1903. Beitrage zur Kenntniss des Cholesterins. (VI. Abhundliung) Monatsh., **24**:648-668.

31. MINOVICI, S.
1908. Beitrage zur Kenntnis des Cholesterins. Ber., **41**:1561-1565.

32. MONTIGNIE, E.
1927. Contribution a l'etude de la cholesterine (2_6 memoire). Bull. soc. chim., **41**:524-527.

33. MONTIGNIE, E.
1927. Contribution a l'etude de la cholesterine (2^e memoire). Bull. soc. chim., soc. chim., **41**:947-949.

34. MONTIGNIE, E.
1928. Contribution a l'etude du cholesterol (5^e memoire). Bull. soc. chim., **43**:1403-1405.

35. MONTIGNIE, E.
1933. Comparaison des reactions du cholesterol avec les acides sulfurique, selenique, tellurique. Bull. soc. chim., **53**:1394.

36. MONTIGNIE, E.
1935. Action de l'iodure mecurique sur le cholesterol. Bull. soc. chim., **2**:1367.

37. MULLER, E., and I. H. PAGE
1933. The preparation of aliphatic cholesteryl ethers and cholesterilene. J. Biol. Chem., **101**:127-132.

38. NORD, F. F.
1919. Uber die katalytische Hydrierung von Cholesterin und Cholesterylen. Biochem. Z., **99**:261-266.

39. OBERMULLER, K.
1891. Beitrage zur Kenntniss des Cholesterins. Z. physiol. chem., **15**:37-48.

40. PICKARD, R. H., and J. YATES
1908. Contributions to the chemistry of the cholesterol group. Part I. The action of hydrogen peroxide and of fused potassium hydroxide on cholesterol. J. Chem. Soc., **93**:1678-1687.

41. PIRRONE, F.
1932. Sulla costituzione chimica della colesterina.—Nota VI. Reazioni della colesterina col pentacloruro di fosforo. Gazz. chim. ital., **62**:63-80.

42. REDTENBACHER, J.
1846. Ueber die Einwirkung der Saltpetersaure auf Choloidinsuare und Cholesterin. Ann., **57**:145-170.

43. ROSENHEIM, O., and H. KING
1934. The ring-system of sterols and bile acids. Part V. On the constitution of ergosterol and its irradiated products. Chemistry & Industry 1934: 196-200; J. Soc. Chem. Ind., **53**:196-200.

44. SCHOENHEIMER, R., and E. A. EVANS, JR.
1936. Allocholesterol and epi-allocholesterol. J. Biol. Chem., **114**:567-582.

45. SKAU, E. L., and W. BERGMANN
1938. The preparation and photochemical oxidation of 2,4-cholestadiene. J. Am. Chem. Soc., **60**:986-987.

46. Stavely, H. E., and W. Bergmann
1937. The chemistry of unsaturated steroids. I. The constitution of cholesterilene. J. Org. Chem., 1:567–574.

47. Stavely, H. E., and W. Bergmann
1937. The chemistry of unsaturated steroids. II. The preparation and properties of 2,4-cholestadiene. J. Org. Chem., 1:575–579.

48. Steinkopf, W., and E. Blummer
1911. Uber einige Ather des Cholesterins. J. prakt. Chem., 84:460–472.

49. Steinkopf, W., H. Winternitz, W. Roederer, and A. Wolynaski
1920. Uber die Kontaktzersetzung des Cholesterins. Ein Beitrag zur Theorie der Erdolbeldung. J. prakt. Chem., 100:65–85.

50. Tchougaeff, L., and W. Fomin
1910. Sur certains derives de la cholesterine. Compt. rend., 150:1435–1437.

Tschugaeff, L., and W. Fomin
1910. Zur Kenntnis des Cholesterins. II. Ann., 375, 288–297.

51. Tschugaeff, L., and A. Gasteff
1909. Zur Kenntnis des Cholesterins. I. Anwendung der Xanthogen-Reaktion. Ber., 42:4631–4634.

52. Tschugaeff, L., and P. Kock
1911. Zur Kenntnis des Cholesterins. III. Ann., 385:352–353.

53. Wagner-Jauregg, T., and L. Werner
1932. Bildung und Unlagerung der Cholesterylather. Z. physiol. Chem., 213:119–124.

54. Walitzky, W. E.
1876. Correspondenzen of the russischem chemischen Gesellschaft. Ber., 9:1310–1311.

55. Walitzky, W. E.
J. russ chem. Ges., 8, 237. Cited in references 3 and 21.

56. Walitzky, W. E.
1881. Sur le cholestene (cholesterilene). Compt. rend., 92:195–196; Ueber das Cholesten. Ber., 14:537–538.

57. Whitby, G. C.
1923. Some new reactions for the detection of sterols. Biochem. J., 17:5–12.

58. Windaus, A.
1906. Ueber Cholesterin. VII. Ber., 39:2249–2262.

59. Windaus, A.
1921. Notizen uber Cholesterin. Z. physiol. Chem., 117:146–158.

60. Windaus, A., and E. Kuhr
1937. Uber die Sulfosauren einiger Sterinabkonnlinge. Ann., 532:52–68.

61. Windaus, A., and R. Seng
1921. Notizen uber cholesterin. IV. Z physiol. Chem., 117:156–158.

62. Wokes, F.
1928. Studies on colour tests for sterols and vitamin A. I. Sterol tests. Biochem. J., 22:830–835.

63. Zelinski, N. D., and N. N. Semiganowsky
1929. Uber die Zersetzung des Cholesterylene und des Cholesterylathers durch Aluminiumchlorid. Ber., 62B:2199–2202.

64. Zwenger, C.
1848. Ueber die chemische Constitution des Cholesterins. Ann., 66:5–13.

65. Zwenger, C.
1849. Ueber die Einwirkung der Phosphorsaure auf Cholesterin. Ann., 69:347–354.

REUTERIA PUTON; FOUR NEW SPECIES FROM THE UNITED STATES (HEMIPTERA, MIRIDAE)

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The genus Reuteria Puton (1875) was originally founded for a new species, *marqueti*, collected in Toulouse, France, by M. Marquet. Reuter (1909) examined specimens of *Capsus irroratus* Say (1832) from North America and pronounced them identical with the European species *Reuteria marqueti* Puton (1875). Van Duzee (1917) in his "Catalogue of Hemiptera" and Oshanin (1909) in "Verzeichnis der Palaearktischen Hemipteren" follow Reuter in this synonomy. The present writer has examined specimens of *Reuteria marqueti* Puton from Europe and finds them different from the five American species considered in this paper.

The genus Reuteria may be distinguished easily from allied genera by the black lines on the basal antennal segment. There are two longitudinal black lines, lateral in position, which connect apically on the ventral aspect. The species are small, pale in color, with green dots, blotches, or tints of green on the hemelytra and femora. The male genital structures give distinctive characters for the species.

Reuteria irrorata (Say)

Capus irroratus Say, Het. Hem. New Harmony, p. 25, 1832.

For many years all specimens of Reuteria have been considered as belonging to a single species, *irrorata* Say. Five species are recognized in the present paper of which one (Pl. I, fig. 3) has been selected as the form most likely described by Say. This species occurs chiefly on elm (*Ulmus americana*) and in addition to the structural characters found in the male genital segment, the hemelytra and dorsal aspect of posterior femora are conspicuously marked with green dots as given in the original description.

Records: IOWA—♂ June 29, 1931, Ames (H. H. Knight). ILLINOIS —♂ June 23, 1918, ♂ ♀ July 12, 1930, Urbana (T. H. Frison), on elm. ♂ ♀ Aug. 11, 1932, Urbana (Knight, Mohr and Ross), on elm. 2 ♂ July 5, 1932, Rockford (Dozier and Mohr). ♂ ♀ July 14, 1932, Starved Rock (Dozier and Park), on elm. MINNESOTA—♀ July 18, 1922, Ramsey Co.; ♂ ♀ July 29, 1919 (H. H. Knight), on elm. NEW YORK—♂ July 29, 1916, ♂ ♀ Aug. 3, 1914, Batavia (H. H. Knight).

Reuteria fuscicornis n, sp.

Distiguished from *irrorata* Say by structure of the male genital claspers (Pl. I, fig. 1) and fuscous coloration of the second antennal segment.

Male. Length 4.3 mm., width 1.3 mm. Head: width .65 mm., vertex .346 mm. Rostrum, length 1.21 mm., reaching to apices of middle coxae. Antennae: segment I, length .43 mm., pale, a heavy black longitudinal line on inner and one on outer margin, the two lines connected apically across

ventral aspect; II, 1.53 mm., blackish at base shading to fuscous on basal half, yellowish apically; III, 1.17 mm., yellowish; IV, .56 mm., dusky. Pronotum: length .52 mm., width at base 1.12 mm. Clothed with simple pale pubescence, hairs longer on prontum, embolium and vertex of head, a few fuscous hairs at tip of clavus; pubescence more recumbent and silky on clavus and corium.

Coloration pale to whitish, hemelytra more translucent; apex and outer basal angle of cuneus, veins at tip of membrane cells, blotch on corium near tip of clavus, bluish green. Differs from *irrorata* Say in having much less green on the hemelytra. Ventral surface and legs pale, femora sometimes shaded with greenish but not forming distinct spots; tibiae with a black point at base.

Female. Length 4.3 mm. Head: width .69 mm., vertex .38 mm. Antennae: segment I, length .43 mm.; II, 1.56 mm.; III, 1.16 mm.; IV, .70 mm.; color similar to the male. Pronotum: length .56 mm., width at base 1.12 mm. Color and pubescence not differing from that of the male.

Holotype: ♂ August 13, 1915, Batavia, New York (H. H. Knight); author's collection. *Allotype:* same data as the type. *Paratypes:* 11 ♂ 2 ♀ taken with the types on hop hornbeam *(Ostrya virginiana)*. NEW YORK — ♂ Aug. 5, 1915, ♂ July 29, 1916, 2 ♀ Aug. 30, 1916, Batavia (H. H. Knight). ♂ July 26, 1916, Ithaca (H. H. Knight). ILLINOIS—74 ♂ ♀ June 25, 1932, Harrisburg (Ross, Dozier and Park), taken on ironwood *(Carpinus caroliniana)*. ♂ ♀ June 23, 1932, Karnak (Ross, Dozier and Park), on *Carpinus caroliniana*. IOWA— ♂ 5 ♀ July 13, 1927, Ames (H. H. Knight). MINNESOTA— ♂ July 6, 1921, St. Anthony Park (H. H. Knight), at light. ONTARIO, CANADA—2 ♂ 2 ♀ July 7, 1916, Parry Sound (H. S. Parish).

Reuteria bifurcata n. sp.

Distinguished from allied species by the bifurcate form of the male genital claspers (Pl. I, fig. 2). Differs from *irrorata* and *fuscicornis* in the absence of green dots on clavus and corium.

Male. Length 4.8 mm., width 1.6 mm. Head: width .75 mm., vertex .36 mm. Rostrum, length 1.3 mm., reaching to middle of intermediate coxae. Antennae: segment I, length .44 mm., black marks typical for the genus; II, 1.69 mm., yellowish, a distinct black ring at base; III, 1.21 mm., yellowish to dusky; IV, .69 mm., dusky yellow. Pronotum: length .62 mm., width at base 1.25 mm. Clothed with pale simple pubescence as in the above species.

Color pale to whitish, hemelytra more translucent, membrane and veins pale; apex of cuneus, a small spot at outer basal angle and tip of embolium dark green. Legs pale, femora without green spots, tibiae with black spot on base.

Female. Length 4.3 mm., width 1.65 mm. Head: width .74 mm., vertex .41 mm. Antennae: segment I, length .47 mm.; II, 1.69 mm.; III, 1.12 mm.; IV, .52 mm. Pronotum: length .60 mm., width at base 1.22 mm. Color and pubescence not differing from that of the male.

Holotype: ♂ July 29, 1916, Batavia, New York (H. H. Knight); author's collection. *Allotype:* same data as the type. *Paratypes:* NEW YORK—4 ♀ taken with the types. 2 ♂ 5 ♀ July 30, 1916, ♂ 4 ♀ July 31, 1916, Batavia (H. H. Knight). MARYLAND— ♂ July 10, 1918, Odonton (W. L. McAtee). OKLAHOMA— ♂ June 21, 1937, Sallisaw (Standish and Kaiser).

Reuteria querci n. sp.

Allied to *irrorata* Say but distinguished by the structure of the male genital claspers (Pl. I, fig. 4). Females may be separated by absence of well formed green blotches on corium and paler veins in the membrane.

Male. Length 4.3 mm., width 1.4 mm. Head: width .71 mm., vertex .35 mm. Rostrum, length 1.17 mm., reaching to near hind margins of middle coxae. Antennae: segment I, length .43 mm., marked with black as typical for the genus; II, 1.51 mm., yellowish, black at base; III, .95 mm., yellowish; IV, .56 mm., yellowish to dusky. Pronotum: length .56 mm., width at base 1.14 mm. Clothed with pale simple pubescence, clavus and corium with somewhat sericeous pubescence, a group of fuscous hairs at tip of clavus.

Color pale to whitish, hemelytra pale to translucent, membrane clear, veins greenish. Margins of cuneus, small dots and blotches on corium and dots on clavus bluish green. The green blotches are smaller and less conspicuous than in *irrorata*. Legs pale, hind femora with some greenish but without well defined blotches; tibial knees black, tips of tarsi fuscous.

Female. Length 4.4 mm., width 1.5 mm. Head: width .70 mm., vertex .36 mm. Antennae: segment I, length .43 mm.; II, 1.51 mm.; III, 1.08 mm.; IV, .21 mm. Pronotum: length .54 mm., width at base 1.08 mm. Coloration and pubescence similar to that of the male.

Holotype: ♂ July 25, 1924, St. Anthony Park, Minnesota (H. H. Knight); author's collection. *Allotype:* taken with the type. *Paratypes:* MINNESOTA—8 ♂ taken with the types on bur oak *(Quercus macro carpa)* which is the host plant of the species. ♀ July 20, 1920, Ramsey Co.; ♂ 2 ♀ Aug. 5, 2 ♀ Aug. 11, 1920, St. Anthony Park (H. H. Knight). ♂ July 20, 1920, Gray Cloud Island (H. H. Knight). IOWA—2 ♂ 2 ♀ July 15, 1932, Ames (Andre and Travis). ILLINOIS— ♀ June 22, Golconda; 2 ♂ 2 ♀ June 23, 1932, Karnak (Ross, Dozier and Park), on Quercus. ♂ June 26, 1934, Alton (DeLong and Ross). ♂ June 23, 1932, Dixon Springs (Ross, Dozier and Park. 3 ♂ July 5, 1932, Rockford (Dozier and Mohr). ♂ June 27, 1932, Urbana (Frison and Ross), on oak. 2 ♂ July 4, 1933, White Heath (H. H. Ross). NEW YORK—2 ♂ 1 ♀ Aug. 10, ♂ 5 ♀ Aug. 12, 1916, ♂ Aug. 5, ♂ Aug. 24, 1915, Batavia (H. H. Knight), on *Quercus macrocarpa.* MARYLAND— ♂ July 29, 1917, Odonton (W. L. McAtee). VIRGINIA— ♂ July 16, Falls Church (N. Banks).

Reuteria pollicaris n. sp.

Distinguished from allied species by the structure of the male genital claspers (Pl. I, fig. 5); the thumblike projections of each clasper suggesting the name. The absence of green spots on corium and clavus is suggestive of *bifurcata* but the fuscous mark bordering the larger areole may serve as a distinction for the separation of females.

Male. Length 3.9 mm., width 1.5 mm. Head: width .71 mm., vertex .37 mm. Rostrum, length 1.56 mm., reaching upon apices of middle coxae. Antennae: segment I, length .39 mm., marked with black lines as typical for the genus; II, 1.43 mm., yellowish, a distinct black ring at base; III, broken. Pronotum: length .56 mm., width at base 1.08 mm. Clothed with simple pale pubescence.

Color pale to whitish, hemelytra semitranslucent, membrane clear, veins green, a distinct fuscous line bordering apex of larger areole; apex of cuneus, a small spot on outer basal angle, and tip of embolium dark

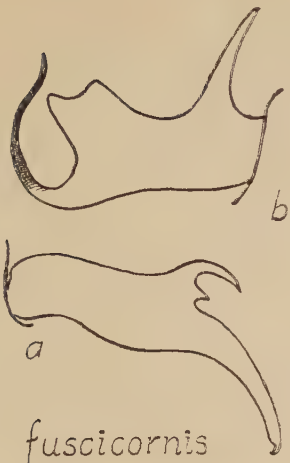
green to fuscous. Legs pale, femora dusky to green on apical half, tibial knees with black spot, tips of tarsi fuscous.

Holotype: ♂ June 25, 1921, Aberdeen, Mississippi (C. J. Drake); author's collection.

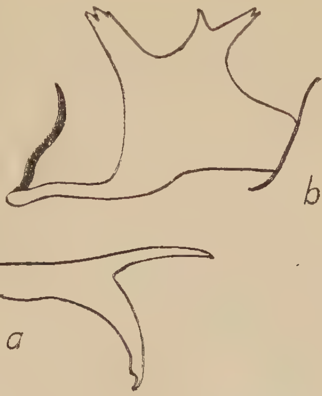
Explanation of Plate I

Male genital claspers of Reuteria species. *a.* left clasper, postero-lateral aspect. *b.* right clasper, postero-lateral aspect.

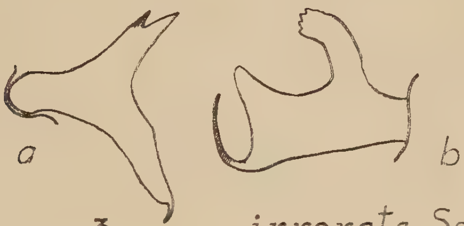
Fig. 1. *Reuteria fuscicornis* n. sp.
Fig. 2. *Reuteria bifurcata* n. sp.
Fig. 3. *Reuteria irrorata* (Say)
Fig. 4. *Reuteria querci* n. sp.
Fig. 5. *Reuteria pollicaris* n. sp.



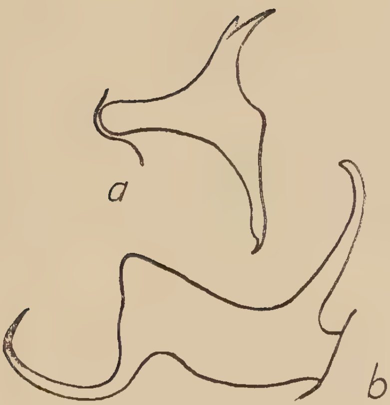
1 *fuscicornis*



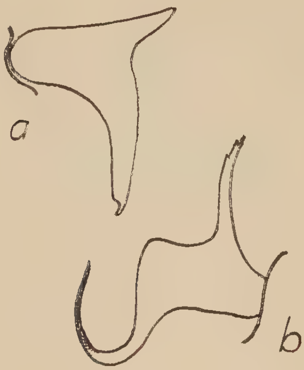
2 *bifurcata*



3 *irrorata* Say



4 *querci*



5 *pollicaris*

HIGH MOLECULAR WEIGHT FATTY ACID DERIVATIVES
I. CHARACTERIZATION OF ACIDS

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Received December 7, 1938

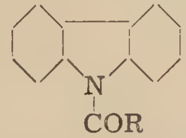
In continuation of studies concerned with animal by-products, an early need was felt for suitable derivatives for the characterization of high molecular weight fatty acids. Several excellent general studies (referred to later in this paper) have been reported on derivatives of acids. Unfortunately, however, derivatives of high molecular weight acids present a somewhat special problem for three reasons. First, the melting points of most derivatives of high molecular weight acids which differ by one or two carbon atoms lie relatively close together. Second, such derivatives of closely related acids do not usually show significant depressions in mixed melting point determinations. Third, derivatives of high molecular weight fatty acids are frequently more difficult to prepare and purify than the derivatives of simple acids.

For these reasons, an examination has been made of new derivatives. It has been found that several of the derivatives prepared are suitable. The acids examined were lauric, myristic, palmitic, stearic and oleic. The tables contained in the Experimental Part give a concise account of the important properties, together with values of the mixed melting points of 50%-50% mixtures of the related, successive members differing by two carbons. The Discussion of Results considers some advantages and disadvantages of the several derivatives.

EXPERIMENTAL PART

All melting point determinations were made by the capillary tube method in an oil bath which was stirred by means of a mechanical stirrer. The same thermometer was used for all melting points and was standardized from time to time. All melting points are uncorrected.

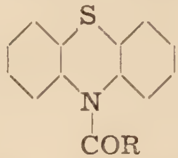
Unless otherwise stated, all acid chlorides used were prepared in a customary manner from the acid and thionyl chloride. Although the acid chlorides were purified by distillation under reduced pressure, equally good results were obtained when the acid chloride was not distilled (subsequent to removal of the excess thionyl chloride).

N-Acylcarbazoles,  .—The reactions for the preparation of these compounds were carried out in a small flask attached to a reflux condenser provided with a calcium chloride tube. To 1.67 g. (0.01 mole) of carbazole was added 0.01 mole of the acid chloride. The mixture was heated by an oil bath at 100-150° until the evolution of hydrogen chloride ceased, and this time was usually one hour or longer.

The black residue which is soluble in the usual organic solvents was crystallized from alcohol, to which decolorizing charcoal had been added. The N-palmitoyl derivative has been reported[1]. The N-acylcarbazoles can be prepared in good yields without the use of potassium carbazole[1]. The oleic acid derivative was an oil. When the N-acylcarbazoles are refluxed for a few minutes in an alcoholic-hydrogen chloride solution, hydrolysis is affected to give carbazole and the fatty acids.

TABLE 1. *N-Acylcarbazoles*

Compound	Melting point	Mixed m.p. with next higher deriv. examined	Anal. percentage N		Pctg. yield
			Calcd.	Found	
N-Lauroylcarbazole	78-79°	68-72°	4.01	3.99	75
N-Myristoylcarbazole	81-82°	73-78°	3.71	3.63	85
N-Palmitoylcarbazole	85-86°	75-78°	3.46	3.60	80
N-Stearoylcarbazole	91-92°		5.23	3.39	83

N-Acylphenothiazines, 

.—The preparation of these compounds from phenothiazine was similar to the method used with the carbazole derivatives. The acyl derivatives crystallized from alcohol as yellow needles. The oleic acid derivative was an oil.

TABLE 2. *N-Acylphenothiazines*

Compound	Melting point	Mixed m.p. with next higher deriv. examined	Anal. percentage N		Pctg. yield
			Calcd.	Found	
N-Lauroylphenothiazine	70°	64-67°	3.68	3.89	78
N-Myristoylphenothiazine	75°	65-68°	3.42	3.65	78
N-Palmitoylphenothiazine	80°	75-78°	3.20	3.50	75
N-Stearoylphenothiazine	86°		3.01	3.30	70

N-Acyl-p-toluenesulfonamides, p-$CH_3C_6H_4SO_2NHCOR$.—The p-toluenesulfonamide and acid chloride, in equivalent quantities, were heated for two hours at 100-125°. Crystallization was effected from alcohol, and again the oleoyl derivative was an oil.

[1] Copisarow, *J. Chem. Soc.*, 113, 816 (1918).

TABLE 3. *N-Acyl-p-toluenesulfonamides*

Compound	Melting point	Mixed m.p. with next higher deriv. examined	Anal. percentage N Calcd.	Anal. percentage N Found	Pctg. yield
N-Lauroyl-*p*-toluene-sulfonamide	83–84°	70–73°	3.97	4.06	50
N-Myristoyl-*p*-toluene-sulfonamide	89–90°	75–78°	3.68	3.77	65
N-Palmitoyl-*p*-toluene-sulfonamide	93–94°	83–85°	3.42	3.49	65
N-Stearoyl-*p*-toluene-sulfonamide	98–99°		3.20	3.36	60

p-*Phenylphenacyl Esters*, p-$C_6H_5C_6H_4COCH_2OCOR$.—These derivatives were prepared in accordance with the directions of Drake[2] from *p*-phenylphenacyl bromide, the acid and sodium carbonate. The esters of lauric, stearic and oleic acids were reported previously[2]. Our *p*-phenylphenacyl ester of stearic acid melted at 97° and not at 91°.

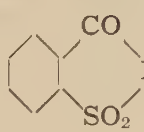
TABLE 4. p-*Phenylphenacyl esters*

Acid	Melting point	Mixed m.p. with next higher deriv. examined	Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Pctg. yield
Lauric	86°	75–78°					92
Myristic	90°	80–85°	79.62	79.80	9.00	9.10	82
Palmitic	94°	85–88°	80.00	80.15	9.31	9.45	91
Stearic	97°		80.33	80.50	9.62	9.70	93
Oleic	60°						

p-*Nitroanilides*, p-$NO_2C_6H_4NHCOR$.—These derivatives were prepared in accordance with the procedure used by Kuhen and McElvain[3], for the corresponding *p*-bromoanilides. Crystallization was from alcohol.

TABLE 5. p-*Nitroanilides*

Acid	Melting point	Mixed m.p. with next higher deriv. examined	Anal. percentage N Calcd.	Anal. percentage N Found	Pctg. yield
Lauric	78°	75–78°	9.27	9.13	65
Myristic	84°	84–86°	8.05	8.08	69
Palmitic	93°	88–91°	7.82	7.79	70
Stearic	96°		6.93	7.00	70

N-Acylsaccharins,  N–COR.—To a suspension of (0.01

[2] Drake and Bronitsky, *J. Am. Chem. Soc.*, 52, 3715 (1930).
[3] Kuhen and McElvain, *ibid.*, 53, 1173 (1931).

mole) of the sodium salt (prepared from saccharin and sodium hydroxide) in 25 cc. of dry chloroform or benzene was added 0.01 mole of the acid chloride. The mixture was refluxed for 3 hours and then filtered hot. Crystallization was from alcohol. The lauric acid derivative is slightly sweet, but the myristic, palmitic and stearic derivatives are not sweet. The oleoyl derivative was an oil.

TABLE 6. *N-Acylsaccharins*

Compound	Melting point	Mixed m.p. with next higher deriv. examined	Anal. percentage N Calcd.	Anal. percentage N Found	Pctg. yield
N-Lauroylsaccharin	88–89°	73–78°	3.84	3.81	60
N-Myristoylsaccharin	90–91°	80–84°	3.56	3.64	76
N-Palmitoylsaccharin	90°	84–87°	3.33	3.49	80
N-Stearoylsaccharin	95°		3.12	3.27	82

2,4-Dinitrophenylhydrazides, 2,4-$(NO_2)_2C_6H_3NHNHCOR$. — These derivatives were prepared in accordance with the procedure applied by Cerezo and Olay[4] to some lower weight fatty acids. After refluxing the benzene solution for 15 minutes, the fine yellow needles were crystallized from alcohol. The stearoyl derivative has been reported[4] as melting at 120°. The oleoyl derivative was obtained as a red viscous oil.

TABLE 7. *2,4-Dinitrophenylhydrazides*

Acid	Melting point	Mixed m.p. with next higher deriv. examined	Anal. percentage N Calcd.	Anal. percentage N Found	Pctg. yield
Lauric	110–111°	95–102°	14.74	14.67	50
Myristic	118°	110–113°	13.72	13.63	60
Palmitic	120–121°	112–115°	12.85	12.73	78
Stearic	123°				75

N-Acyl-2-nitro-p-toluidides, $(2\text{-}NO_2)(4\text{-}CH_3)C_6H_3NHCOR$. — The acid chloride and 2-nitro-*p*-toluidine (in equivalent quantities, as usual) were heated for 3 hours at 100-150°. The yellow crystals were obtained from alcohol.

TABLE 8. *N-Acyl-2-nitro-*p*-toluidides*

Compound	Melting point	Mixed m.p. with next higher deriv. examined	Anal. percentage N Calcd.	Anal. percentage N Found	Pctg. yield
N-Lauroyl-2-nitro-*p*-toluidide	62–63°	58–60°	8.38	8.26	83
N-Myristoyl-2-nitro-*p*-toluidide	73–74°	65–68°	7.73	7.92	82
N-Palmitoyl-2-nitro-*p*-toluidide	78–79°	72–74°	7.18	7.25	80
N-Stearoyl-2-nitro-p-toluidide	85°		6.70	6.90	75

[4] Cerezo and Olay, *Anales Soc. españ. fis. quim.*, 32, 1090 (1934).

p-Tolylmercuric salts, *p*-$CH_3C_6H_4HgOCOR$. — A mixture of 0.005 mole of di-*p*-tolylmercury, 0.005 mole of the acid and 25 cc. of xylene was refluxed for 8 hours. The fine crystals were obtained from alcohol. Each derivative when heated with hydrochloric acid gave the corresponding acid, toluene, and mercuric chloride. The oleic acid derivative was a viscous oil.

TABLE 9. p-*Tolylmercuric salts*

Acid	Melting point	Mixed m.p. with next higher deriv. examined	Anal. percentage Hg Calcd.	Anal. percentage Hg Found	Pctg. yield
Lauric	93–94°	80–85°	40.76	40.40	65
Myristic	95–96°	84–89°	38.56	38.20	65
Palmitic	99°	85–92°	36.70	36.27	75
Stearic	102–103°		34.91	34.40	60

Phenylmercuric Salts, $C_6H_5HgOCOR$.—These derivatives were prepared after the method used with the *p*-tolylmercury salts, but the period of refluxing in xylene was 5 hours. Boiling hydrochloric acid cleaved the compounds to the corresponding acid, benzene and mercuric chloride. Phenylmercuric myristate was prepared earlier[5] by heating to 120° a mixture of myristic acid and diphenylmercury in alcohol. The oleoyl derivative was obtained as a viscous oil.

TABLE 10. *Phenylmercuric salts*

Acid	Melting point	Mixed m.p. with next higher deriv. examined	Anal. percentage Hg Calcd.	Anal. percentage Hg Found	Pctg. yield
Lauric	82°	68–73°	41.96	41.50	50
Myristic	86°	75–78°	39.63	39.30	50
Palmitic	93°	80–85°	37.66	37.50	60
Stearic	95°		35.78	35.40	71

Triphenyllead Salts, $(C_6H_5)_3PbOCOR$.—The solution of 0.005 mole tetraphenyllead and 0.005 mole of acid in 25 cc. of xylene was refluxed for 10 hours. In this way, only a very small amount of tetraphenyllead was recovered, but more tetraphenyllead was recovered when the period of refluxing was only 5 hours. Crystallization was from alcohol. When hydrogen chloride was passed into chloroform solutions of the compounds, a precipitate of diphenyllead dichloride formed immediately. The fatty acids were also isolated in the cleavage products. Analysis for lead was by the gravimetric method[6] used in related studies.

Under corresponding conditions, there was no reaction between tetraphenyltin and stearic acid; no reaction after refluxing in xylene for 10 hours with silica gel[7] as a catalyst; and no reaction after heating tetraphenyltin and stearic acid in 95% alcohol for 6 hours at 150-180° in a

[5] Otto, *J. prakt. Chem.*, [2] **1**, 185 (1870).

[6] Gilman and Robinson, *J. Am. Chem. Soc.*, **50**, 1714 (1928).

[7] Browne and Reid, *ibid.*, **49**, 830 (1927).

sealed tube. These negative results confirm the relative reactivities[8] of organotin and organolead compounds.

TABLE 11. *Triphenyllead salts*

Acid	Melting point	Mixed m.p. with next higher deriv. examined	Anal. percentage Pb Calcd.	Anal. percentage Pb Found	Pctg. yield
Lauric	91°	82–84°	32.53	32.90	89
Myristic	102–103°	94–100°	30.44	30.75	60
Palmitic	110°	108–110°	29.89	29.40	55
Stearic	112°		28.73	28.74	50

Monoureides, $H_2NCONHCOR$.—The monoureide of stearic acid was first prepared in accordance with the procedure of Stendal[9] from urea, ethyl stearate, and sodium ethylate in pyridine. Better yields of the monoureides were then obtained by refluxing for 3 hours a mixture of 0.01 mole of urea, 0.01 mole of acid chloride and 25 cc. of dry pyridine. Crystallization was affected from an alcohol-acetic acid solution. Also, the stearoylurea was prepared in a 30% yield by refluxing urea and stearoyl chloride in dry benzene for 6 hours. The ureides of stearic[9, 10] and oleic[9] acids have been reported.

TABLE 12. *Monoureides*

Acid	Melting point	Mixed m.p. with next higher deriv. examined	Anal. percentage N Calcd.	Anal. percentage N Found	Pctg. yield
Lauric	182°	175–177°	11.60	11.86	66
Myristic	178°	173–175°	10.37	10.66	60
Palmitic	175°	170–173°	9.39	9.56	70
Stearic	174°				60
Oleic	160°				50

Monothioureides, $H_2NCSNHCOR$.—These derivatives were prepared like the ureides from thiourea and the acid chloride in pyridine. Crystallization was from alcohol.

Distearoylthiourea, $C_{17}H_{35}CONHCSNHCOC_{17}H_{35}$, was prepared by adding a mixture of 22 cc. of 25% sodium ethylate, 7 cc. pyridine, and 1.44 g. (0.02 mole) of thiourea to 4 g. of ethyl stearate. After 24 hours the mixture was added to dilute acetic acid to give a 50% yield of crude product, which when crystallized from alcohol melted at 100°.

Anal. Calcd. for $C_{37}H_{72}O_2N_2S$: N, 4.61. Found: N, 4.81 and 4.61.

[8] Gilman and Nelson, *Rec. trav. chim.*, 55, 518 (1936).

[9] Stendal, *Compt. rend.*, 196, 1810 (1933).

[10] Jacobson, *J. Am. Chem. Soc.*, 58, 1984 (1936).

TABLE 13. *Monothioureides*

Acid	Melting point	Mixed m.p. with next higher deriv. examined	Anal. percentage N Calcd.	Anal. percentage N Found	Pctg. yield
Lauric	138°	132–136°	10.85	10.82	85
Myristic	135°	128–130°	9.78	9.99	70
Palmitic	135–136°	128–130°	8.91	8.93	71
Stearic	133°		8.18	8.19	85
Oleic	112–113°		8.23	8.35	40

p-*Xenylamides*, p-$C_6H_5C_6H_4NHCOR$.—These derivatives were prepared by treating equimolecular quantities of the acids and *p*-xenylamine in a sealed tube for 5 hours at 135-140°, and crystallizing from alcohol, or better from ethyl acetate[11]. Equally good yields were obtained when the amine and acid chloride were heated for 5 hours at 150-200°.

TABLE 14. p-*Xenylamides*

Acid	Melting point	Mixed m.p. with next higher deriv. examined	Anal. percentage N Calcd.	Anal. percentage N Found	Pctg. yield
Lauric	146°	135–140°	3.98	3.84	45
Myristic	143°	133–135°	3.69	3.76	40
Palmitic	142°	137–139°	3.44	3.34	55
Stearic	143°		3.22	3.26	60

p-*Acylbiphenyls*, p-$C_6H_5C_6H_4COR$.—Each of these derivatives was prepared by a Friedel-Crafts reaction in carbon disulfide. Crystallization was from ethyl acetate. The position of the acyl group in the stearoyl derivative was shown[12] to be *para* by comparison with an authentic specimen prepared from *p*-phenylphenylmagnesium bromide and stearonitrile. It was assumed that the same orientation applied to the other acyl derivatives. A preliminary experiment indicated that the oleoyl ketone probably was tied up as an aluminum chloride complex[13].

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[11] Kimura and Nihayashi, *Ber.*, **68B**, 2028 (1935).

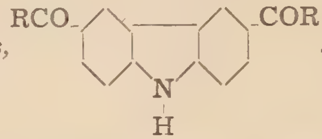
[12] Studies by M. R. McCorkle. See, also, Ralston and Christensen, *Ind. Eng. Chem.*, **29**, 194 (1937).

[13] Gangloff and Henderson, *J. Am. Chem. Soc.*, **39**, 1420 (1917).

TABLE 15. p-*Acylbiphenyls*

Compound	Melting point	Mixed m.p. with next higher deriv. examined	Anal. percentage C and H				Pctg. yield
			Carbon		Hydrogen		
			Calcd.	Found	Calcd.	Found	
Lauroyl-biphenyl	101–102°	90–94°	85.71	85.90	9.52	9.60	77
Myristoyl-biphenyl	102–103°	96–98°	85.71	85.60	9.89	9.98	73
Palmitoyl-biphenyl	103–104°	97–99°	85.71	85.85	10.20	10.50	76
Stearoyl-biphenyl	106–107°		85.71	85.50	10.48	10.70	75

2,8-Diacylcarbazoles,



.—The Friedel-Crafts reactions were carried out with 0.005 mole of carbazole, 0.01 mole of acid chloride, 0.02 mole of aluminum chloride and 25 cc. of nitrobenzene. The mixture was stirred for 12 hours at room temperature and then worked up in a customary manner. The 2,8-distearoylcarbazole was prepared earlier[12]. Although the positions of the acyl groups were not established experimentally, there is no doubt that they are 2 and 8, inasmuch as the corresponding diacyl compound[14] (prepared analogously) was shown to have the acyl groups in positions 2 and 8.

TABLE 16. *2,8-Diacylcarbazoles*

Compound	Melting point	Mixed m.p. with next higher deriv. examined	Anal. percentage N		Pctg. yield
			Calcd.	Found	
2,8-Dilauroylcarbazole	176°	155–160°	2.50	2.70	59
2,8-Dimyristoylcarbazole	169°	158–163°	2.39	2.57	50
2,8-Dipalmitoylcarbazole	162°	160–162°	2.28	2.47	65
2,8-Distearoylcarbazole	163°		2.18	2.30	65

p-*Acylaminobenzoic Acids*, *p*-$HOOCC_6H_4NHCOR$.—Reactions were carried out by refluxing for 5 hours a mixture of 0.01 mole of *p*-aminobenzoic acid, 0.01 mole of acid chloride and 25 cc. of dry pyridine. Purification was effected by crystallization from acetic acid.

[14] Plant, Rogers and Williams, *J. Chem. Soc.*, 741 (1935).

TABLE 17. p-*Acylaminobenzoic acids*

Compound	Melting point	Mixed m.p. with next higher deriv. examined	Anal. percentage N		Pctg. yield
			Calcd.	Found	
p-Lauroylaminobenzoic acid	227–228°	225–227°	4.39	4.53	80
p-Myristoylaminobenzoic acid	224–225°	222–225°	4.03	4.19	72
p-Palmitoylaminobenzoic acid	226–227°	220–224°	3.73	3.80	60
p-Stearoylaminobenzoic acid	221°		3.47	3.72	75

N-Palmitoyl- and N-Stearoylanthranilic Acids, o-$HOOCC_6H_4NHCOR$.—These derivatives were prepared by refluxing for 5 hours a mixture of 0.01 mole of anthranilic acid, 0.01 mole of the acid chloride and 25 cc. of dry chloroform. Crystallization was from alcohol.

The N-palmitoylanthranilic acid melted at 100°.

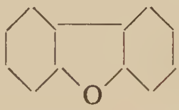
Anal. Calcd. for $C_{23}H_{37}O_3N$: N, 3.37. Found: N, 3.90.

The N-stearoylanthranilic acid melted at 113°.

Anal. Calcd. for $C_{25}H_{41}O_3N$: N, 3.47. Found: N, 3.65.

A mixed melting point determination of a 50% mixture of the two acids melted at 90-93°. The oils obtained from reactions with lauroyl and myristoyl chlorides have not yet crystallized.

3-Palmitoyl- and 3-Stearoylaminodibenzofuran,



NHCOR.—

A mixture of 0.01 mole of 3-aminodibenzofuran and 0.01 mole of the acid chloride was heated for 5 hours at 125-160°. Crystallization was from acetone.

The 3-palmitoylaminodibenzofuran melted at 130°.

Anal. Calcd. for $C_{28}H_{39}O_2N$: N, 3.32. Found: N, 3.33.

The 3-stearoylaminodibenzofuran melted at 134°.

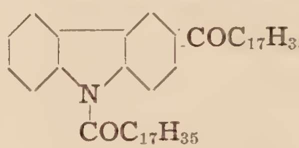
Anal. Calcd. for $C_{30}H_{43}O_2N$: N, 3.12. Found: N, 3.30.

A mixed melting point determination of a 50% mixture of the two compounds melted at 130°.

Sym.-*Diacylbenzidines,* $RCONHC_6H_4C_6H_4NHCOR$. — These derivatives were prepared by refluxing for 5 hours a mixture of 0.005 mole of benzidine, 0.01 mole of the acid chloride and 25 cc. of dry pyridine. Purification was effected by crystallizing from pyridine. These derivatives are insoluble in the ordinary organic solvents; and although they are slightly soluble in hot acetic acid they do not crystallize well from this solvent.

TABLE 18. Sym.-*Diacylbenzidines*

Compound	Melting point	Mixed m.p. with next higher deriv. examined	Anal. percentage N Calcd.	Anal. percentage N Found	Pctg. yield
Dilauroylbenzidine	248°	238–240°	5.10	5.28	84
Dimyristoylbenzidine	241–242°	230–233°	4.63	4.80	83
Dipalmitoylbenzidine	233°	226–228°	4.24	4.40	77
Distearoylbenzidine	232°		3.91	4.00	93

N-Stearoyl-2-stearoylcarbazole,  .—First, 2-stearoylcarbazole[12] was prepared from carbazole, stearoyl chloride and aluminum chloride in nitrobenzene. The yield was 60% and the compound melted at 103-105° after crystallization from alcohol. Then, 0.01 mole of 2-stearoylcarbazole and 0.01 mole of stearoyl chloride was heated at 150-200° until hydrogen chloride was no longer evolved. Crystallizations from acetone and then from ethyl acetate gave 2.5 g. of compound melting at 86-87°.

Anal. Calcd. for $C_{48}H_{77}O_2N$: N, 2.03. Found: N, 2.25 and 2.30.

When a Friedel-Crafts reaction was carried out between 0.01 mole of N-stearoylcarbazole, 0.02 mole of stearoyl chloride and 0.02 mole of aluminum chloride in 40 cc. of nitrobenzene, there resulted a 45% yield of 2,8-distearoylcarbazole which was identified by the method of mixed melting points. Hydrolysis of N-stearoyl-2-stearoylcarbazole by refluxing with alcoholic-hydrochloric acid for 4 hours yielded 2-stearoylcarbazole and stearic acid.

Under corresponding conditions there was no evidence of significant rearrangement of 1-stearoylaminonaphthalene[15], $C_{10}H_7NHCOC_{17}H_{35}$. That is, the N-acyl group in naphthalene did not rearrange to the C-acyl group as was the case with carbazole, instead most of the 1-stearoylamino-naphthalene was recovered. Of course, we may not be dealing here with a true rearrangement but rather with a cleavage followed by acylation.

DISCUSSION OF RESULTS

Potentially there is a possibility of having a staggering number of acid derivatives. Actually there are very many acid derivatives in a broad sense, because the carboxyl group in acids has lent itself to the preparation of many derivatives which crystallize well and have sharp melting points. However, only scattered studies have been carried out on a series of acids with a selected reactant with a view to obtaining a broadly useful derivative. Limitation of space precludes a full account of such studies. Some of the more extensive and also more recent investigations have been made by Reid, Kelly, Drake, De'Conno, Robertson, McElvain, Ralston, Brauns, Cerezo, Tucker, Donleavy, Pollard and their co-workers.

[15] De'Conno, *Gazz. chim. ital.*, 47, *I*, 93 (1917) [*C.A.*, 12, 1172 (1918)].

Among the derivatives which have been examined more systematically are the *p*-nitrobenzyl esters[16]; substituted and unsubstituted phenacyl esters[17]; substituted amides[18]; imidazoles[19]; substituted hydrazides[20]; S-benzyl thiouronium salts[21]; piperazides[22]; and monoureides[23].

Some specific comments on the series of derivatives now reported by us follow. The N-acylcarbazole derivatives appear to be the most satisfactory for identifying lauric, myristic, palmitic and stearic acids. These derivatives require a relatively short time to prepare and give good yields of an easily purified crystalline product. They show a difference in melting points of adjacent members of from 3-5°, and a depression of mixed melting points of from 4-8°.

The N-acyl-*p*-toluenesulfonamides are quite stable. These derivatives show an even larger depression in mixed melting points than the carbazole derivatives, varying from 8 to 11°. They crystallize well from alcohol or acetone, and only two or three crystallizations are necessary for purification.

The *p*-phenylphenacyl esters have proven satisfactory derivatives for a large number of acids[17]. They have one decided advantage in that they are prepared from the sodium salt of the acid. The derivatives of lauric, myristic, palmitic and stearic acids are colorless crystals.

The phenothiazine derivatives are similar to the carbazole derivatives, however, they are not so satisfactory. The do crystallize well, but are somewhat lower melting and show a smaller depression in mixed melting points than the corresponding carbazole derivatives.

The N-acyl-2-nitro-*p*-toluidine derivatives are much better derivatives than a large number of those which are listed in the literature. However, they show a smaller difference in melting points between successive compounds than some derivatives which are described in this report.

The saccharin derivatives are quite stable, and they are readily prepared from the sodium salt of saccharin and the acid chloride in some inert solvent, such as chloroform or benzene.

In this study, the 2,4-dinitrophenylhydrazides are the most easily prepared derivatives in terms of time consumed. These derivatives are

[16] Reid and co-workers, *J. Am. Chem. Soc.*, 39, 124, 701, 1727 (1917); Kelly and Segura, *ibid.*, **56**, 2497 (1934); Blicke and Smith, *ibid.*, **51**, 1947 (1929).

[17] Reid and co-workers, *ibid.*, 41, 75 (1919); 42, 1043 (1920); 43, 629 (1921); 52, 818 (1930); 54, 2101 (1932); Kelly and Kleff, *ibid.*, 54, 4444 (1932); Drake and Bronitsky, *ibid.*, 52, 3715 (1930); Drake and Sweeney, *ibid.*, 54, 2059 (1932); Wrede and Rathaas, *Ber.*, **67B**, 739 (1934).

[18] De'Conno (Ref. 15); Robertson, *J. Chem. Soc.*, **115**, 1210 (1919); Kuhen and McElvain, *J. Am. Chem. Soc.*, 53, 1173 (1931); D'Alelio and Reid, *ibid.*, 59, 109, 111 (1937); Carré and Libermann, *Bull. soc. chim.*, 53, 293 (1933); Hardy, *J. Chem. Soc.*, 398 (1936); Tucker, *J. Am. Chem. Soc.*, 57, 1989 (1935); Birosal and Huang, see *C.A.* 27, 5728 (1933); Escher, *Helv. Chim. Acta*, 12, 27 (1929); Kimura and Hihayashi, *Ber.*, **63B**, 2028 (1935).

[19] Pool, Harwood and Ralston, *J. Am. Chem. Soc.*, **59**, 178 (1937).

[20] Brauns, *ibid.*, 42, 1478 (1920); Vesely and Haas, *Chem. Listy*, **21**, 351 (1927); [*C.A.*, 22, 58 (1928)]; Cerezo and Olay, *Anales soc. españ. fis. quim.*, **32**, **1090** (1934).

[21] Donleavy, *J. Am. Chem. Soc.*, **58**, 1004 (1936).

[22] Pollard and co-workers, *ibid.*, **56**, 150, 1759 (1934).

[23] Stendal, *Compt. rend.*, **196**, 1810 (1933); Jacobson, *J. Am. Chem. Soc.*, **58**, 1984 (1936).

easily crystallized and give well-defined yellow needles. Only one or two crystallizations are necessary to obtain a pure product. The one disadvantage to these derivatives is that the melting points do not exhibit as wide a range as desired. However, this is somewhat obviated by the use of mixed melting points. Even though the stearoyl and palmitoyl derivatives show only 2° difference in melting points there is a lowering of 8° in mixed melting points.

The *p*-xenylamides have high melting points, relatively speaking, but exhibit a very small difference in melting points between adjacent members. This disadvantage is not overcome by mixed melting points.

The monoketones and diketones (prepared by attaching one or two acyl groups to carbon) are not very satisfactory derivatives. They are more difficult to prepare than most of the previously mentioned derivatives and do not form well-defined crystalline compounds. This is especially true of the diketones which are non-crystalline and are very difficult to purify. However, these disadvantages would not be so great provided the compounds showed a wide range in melting points and a large depression in mixed melting points. Unfortunately, this is not the case.

The ureides and thioureides have well-defined physical properties which make purification and analysis most convenient. These compounds are easily prepared in good yields but have the disadvantage that they show very little variation in melting points. Mixed melting point determinations do not remove this difficulty.

The mercury and lead salts have the disadvantage of being relatively inaccessible. More particularly they show no exceptional properties as derivatives, in fact they are much less satisfactory than several derivatives previously mentioned in this study.

The N-acylanthranilic acid derivatives have excellent properties in so far as melting points are concerned. However, they are difficult to prepare and purify; a large number of crystallizations are necessary for purification. The lower members were oils.

Considering the ease of preparation and purification as well as the range in melting points and depression of mixed melting points, the ten best series of derivatives in this study may be listed in the following order of decreasing importance; (1) the N-acylcarbazole derivatives; (2) the N-acyl-*p*-toluenesulfonamides; (3) the *p*-phenylphenacyl esters; (4) the N-acylphenothiazine derivatives; (5) the N-acyl-2-nitro-*p*-toluidine derivatives; (6) the N-acylsaccharin derivatives; (7) the 2,4-dinitrophenylhydrazides; (8) the *p*-nitroanilides; (9) the phenylmercuric salts; (10) the *p*-xenylamides.

The results of the present study assist in making available several useful types of derivatives, and there are occasions when complete characterization warrants the preparation of more than one derivative. It is, of course, not certain that some of the new types now described can be used for derivativizing satisfactorily other lower or higher acids, normal or branched.

Acknowledgments. The authors are grateful to Dr. A. W. Ralston for helpful suggestions as well as for liberal supplies of stearic acid. Thanks are also due Miles R. McCorkle for assistance.

SUMMARY

Several derivatives useful for the characterization of lauric, myristic, palmitic and stearic acids have been described.

STUDIES ON THE FAT OF CHEDDAR CHEESE[1]

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Studies on flavor development in cheddar cheese have included primarily changes in the proteins. The formation and accumulation of various compounds through breakdown of the casein complexes often have been considered responsible for the typical "cheddar" flavor. Changes in the fat have been investigated relatively little.

The fats of cheeses other than cheddar have received some attention. Orla-Jensen (5) studied hydrolysis of the fats in various cheeses and related the products formed to certain cheese flavors. Windisch (7) noted changes in the fats of four cheeses made in Germany and suggested that enzymes produced by certain bacteria hydrolyzed the fats with formation of free fatty acids; a portion of these acids disappeared with continued ripening of the cheeses. Currie (2) associated the typical flavor of roquefort cheese with formation of certain fatty acids and their salts from the fat. Lane and Hammer (4) found progressive increases in the acid numbers of the fat of blue (roquefort type) cheese as the cheese ripened. In recovering certain flavor constituents from cheddar cheese by distillation, Suzuki, Hastings and Hart (6) suggested the possibility of fat hydrolysis accounting for the relatively large amounts of fatty acids obtained.

In experiments with cheddar cheese at the Iowa Agricultural Experiment Station, fat and serum from ripened cheese often were recovered by pressure (3). After these materials had been separated and filtered, they were examined frequently for taste and aroma. With ripened cheese, both the fat and the serum had a flavor suggestive of the original product. The fat regularly was distinctly cheesy; the serum likewise suggested cheese (3) although it also was comparable in flavor to certain protein decomposition products. Since it appeared that flavor constituents of cheddar cheese are contained in the fat, additional studies on the fat seemed desirable.

ACID NUMBERS OF CHEDDAR CHEESE FAT

Fat was recovered from cheese by pressure (3), a mixture of 400 gm. of finely divided cheese and 600 gm. of very fine sand commonly being used, and was separated from the accompanying serum in a separatory funnel and then filtered through paper. The acids in the fat were titrated with the alcoholic potassium hydroxide method suggested by Breazeale and Bird (1). For comparison, fat was recovered from butter by melting the butter and filtering the fat through paper and from blue cheese by the method used with cheddar cheese. Table 1 presents acid numbers of fats from 20 samples of cheddar cheese, 5 of normal, sweet cream butter, 5 of rancid butter, and 5 of blue cheese; the values are recorded as milliliters of 0.1N. potassium hydroxide required to neutralize 10 gm. of fat.

The acid numbers of fats from cheddar cheese were definitely higher than those of fats from normal, sweet cream butter and approached those

[1] Journal Paper J-601 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 385.

TABLE 1. *Acid numbers of fats from cheddar cheese and other dairy products*

Sample no.	Source of product	Milk or cream used	Approximate age of product	Acid no. as ml. 0.1N. KOH per 10 gm. fat
		Cheddar cheese		
1	Wisconsin	Raw	Well aged	2.15
2	Wisconsin	Raw	Well aged	2.65
3	Wisconsin	Raw	18 mo.	2.6
4	Wisconsin	Raw	19 mo.	2.15
5	Canada	Raw	19 mo.	3.5
6	Wisconsin	Raw	Well aged	2.6
7	New York	Raw	Well aged	5.9
8	Wisconsin	Raw	Well aged	3.2
9	Wisconsin	Raw	24 mo.	3.6
10	Wisconsin	Raw	7 mo.	1.8
11	Wisconsin	Raw	24 mo.	3.2
12	New York	Raw	36 mo.	9.6
13	Iowa	Raw	8 mo.	2.4
14	Iowa	Past.	6 mo.	1.7
15	Iowa	Past.	8 mo.	1.5
16	Iowa	Past.	14 mo.	1.75
17	Iowa	Past.	14 mo.	1.5
18	New Zealand		48 mo.	4.4
19	Iowa	Past.	22 mo.	1.9
20	Washington	Past.	24 mo.	2.3
		Normal, sweet cream butter		
1	Iowa	Past.	1 da.	0.4
2	Iowa	Past.	1 da.	0.5
3	Iowa	Past.	2 da.	0.45
4	Iowa	Past.	2 da.	0.55
5	Iowa	Past.	2 da.	0.5
		Rancid butter*		
1	Iowa	Past.	10 da.	2.5
2	Iowa	Past.	10 da.	2.35
3	Iowa	Past.	10 da.	4.0
4	Iowa	Past.	10 da.	9.7
5	Iowa	Past.	10 da.	7.9
		Blue (roquefort type) cheese		
1	Wisconsin	Raw	12 mo.	22.0
2	Iowa	Raw**	5 mo.	25.5
3	Iowa	Past.**	5 mo.	16.0
4	Denmark		13 mo.	27.0
5	France		14 mo.	23.2

* Made from pasteurized sweet cream inoculated with lipolytic organisms.
** homogenized.

of fats from butter that was definitely rancid. They were very low compared to values on fats from blue cheese since this type requires extensive fat hydrolysis by the mold for development of the characteristic flavor. The acid numbers of fats from raw milk cheese were usually higher than those of fats from pasteurized milk cheese. In general, the results suggest a change in the acid number of fat of cheddar cheese during ripening, whether raw or pasteurized milk is employed.

CHANGES IN ACID NUMBER OF FAT AND IN VOLATILE ACIDITY OF CHEDDAR CHEESE DURING RIPENING

Changes in acid number of the fat and in volatile acidity of cheddar cheese during ripening were studied with six lots of cheese. For each lot, 510 pounds of milk, testing about 3.8 per cent fat, was thoroughly mixed and divided into two equal portions; one portion was pasteurized at 62.8°C. for 30 minutes. The two portions of milk were made into longhorns with the methods followed in earlier studies (3). After 2, 90 and 200 days of ripening, the acid numbers of the fats and the volatile acidities were determined on each type of cheese. The method used for the volatile acidities consisted of distilling with steam a mixture of 400 gm. of cheese and 100 ml. 1N. sulfuric acid and titrating the first liter of distillate with 0.1N. sodium hydroxide; the results are expressed as milliliters of the alkali required. With only 1 liter of distillate not all the volatile acids are recovered so that the values are useful primarily for comparative purposes. The acid numbers and volatile acidities, together with data on the cheese flavors, are presented in table 2.

The acid numbers of fats from fresh cheese (2 days old) were relatively high compared to the values on fats from sweet cream butter (table 1). As ripening progressed the values increased, whether the cheese was from raw or pasteurized milk. The acid numbers of fats from

TABLE 2. *Changes in acid number of fat and volatile acidity of cheddar cheese during ripening*

Sample no.	Milk used	Acid no. as ml. 0.1N. KOH per 10 gm. fat			Total vol. acidity as ml. 0.1N. NaOH per 400 gm. cheese			Flavor of cheese after 200 days
		2 days	90 days	200 days	2 days	90 days	200 days	
1-1	Raw	1.2	1.8	2.4	18.4	38.7	36.4	V.* good, sharp
1-2	Past.	1.0	1.2	1.45	10.3	16.0	22.2	Good, mild
2-1	Raw	1.3	1.6	2.2	15.3	40.1	35.2	V. good, sharp
2-2	Past.	1.0	1.1	1.3	9.5	14.3	23.8	Good, mild
3-1	Raw	1.25	1.6	2.15	17.5	32.5	31.0	V. good, sharp
3-2	Past.	0.9	1.1	1.15	11.2	16.5	23.5	Good, sl.† lacking
4-1	Raw	1.0	1.3	2.25	12.0	39.8	37.1	Sharp, sl. bitter
4-2	Past.	0.75	0.9	1.1	10.0	15.2	33.6	Good, sl. lacking
5-1	Raw	1.0	1.3	2.45	12.8	44.8	28.5	Sharp, sl. bitter
5-2	Past.	0.8	0.85	1.05	12.7	25.3	28.2	Sl. sour, sl. lacking
6-1	Raw	1.05	1.5	2.35	12.7	32.1	35.7	V. sharp, sl. ferm.‡
6-2	Past.	0.85	1.0	1.15	9.0	16.0	21.3	Sl. sour, sl. lacking

* V = very. † sl. = slightly. ‡ ferm. = fermented.

raw milk cheese were slightly higher when the cheese was fresh and also showed greater increases than those of fats from pasteurized milk cheese so that at the end of the ripening the former were roughly twice the latter. The volatile acidities of raw milk cheese were regularly higher than those of pasteurized milk cheese. In five trials the values for raw milk cheese increased up to 90 days and then decreased, although in most instances the decrease was small, while in the remaining trial the value increased during the entire ripening, with only a small increase from 90 to 200 days. With pasteurized milk cheese the values regularly increased as the ripening progressed. In general, the flavors of all the cheese were considered satisfactory after 200 days, regardless of whether raw or pasteurized milk was employed. Raw milk cheese regularly had considerable of the "sharp" flavor typical of aged cheddar cheese, whereas pasteurized milk cheese developed comparatively little of the "sharp" flavor and usually lacked flavor.

DISTILLATION OF FLAVORING MATERIALS FROM CHEDDAR CHEESE

Attempts were made to obtain flavoring materials from cheddar cheese by distillation with steam. Twenty samples, 14 from raw milk and 6 from pasteurized milk, were used. They included cheese of various ages made in Iowa, New York, Washington and Wisconsin. With each sample 400 gm. of finely divided cheese was acidified with 50 ml. 1N. sulfuric acid in a flask, and the mixture distilled with steam until 1 liter of distillate was obtained. This was practically saturated with sodium chloride, about 100 ml. of ethyl ether added, and the mixture shaken occasionally during about 24 hours. The ether layer was removed with a separatory funnel and anhydrous sodium sulfate added. After shaking occasionally during 48 hours the ether was filtered and allowed to evaporate slowly from a narrow-neck flask at room temperature; the flask was then stoppered.

A small amount (usually a few drops) of a brown, oily liquid was left in each flask. The residues varied considerably in aroma but all were described as cheesy, even by persons unfamiliar with the source. The aromas suggested some of the higher volatile fatty acids, although certain of the residues apparently contained other aroma materials. Larger amounts of residue were obtained from raw milk cheese than from pasteurized milk cheese, and the aromas of the former were the more suggestive of aged cheese.

To verify the presence of acids in the residues, neutral ethyl alcohol was added to several, the mixtures boiled, and then titrated with 0.1N. sodium hydroxide, using phenolphthalein. One residue required 16.2 ml. of the alkali to neutralize it while others required from 3.8 to 9.6 ml. Although these values are relatively small, not all the volatile acid was distilled from the cheese and there undoubtedly was a loss during the recovery with ether. Very little of the higher volatile fatty acids would be required to influence the flavor of a product like cheese.

DISTILLATION OF FLAVORING MATERIALS FROM CHEDDAR CHEESE FAT

Attempts were made to distill flavoring materials from cheddar cheese fat. About 150 gm. of fat was pressed from each of three well-aged cheese, and each lot was distilled with steam until 500 ml. of distillate was collected. The distillates had cheesy aromas. The residue recovered by ex-

tracting a distillate with ether was similar to those obtained previously from cheese. The aroma definitely suggested cheese and higher volatile fatty acids appeared to be involved.

DISTRIBUTION OF FATTY ACIDS IN BUTTER FAT AND IN SODIUM CHLORIDE SOLUTION

Since fat of cheddar cheese appeared to contain small amounts of higher volatile fatty acids, the solubility of fatty acids in fat was studied. About 0.2 ml. of a fatty acid was added to a mixture of 20 ml. of melted fresh butterfat and 20 ml. of 4 per cent sodium chloride solution in a flask, salt solution being used rather than water to make conditions more comparable to cheese. The mixture was shaken until the fat solidified and then held (well stoppered) 7 days at about 7.2°C. The fat was melted on a water bath, the fat and salt solution separated, each filtered through paper, and then 10 ml. of each was titrated with 0.1N. alkali; for the fat the usual procedure (1) was followed. Table 3 gives the amounts and percentages of acids retained in the fat and in the salt solution with each of 8 fatty acids.

The data show that, as would be expected, the solubility of fatty acids in butter fat increased as the molecular weights of the acids increased, whereas in the salt solution the reverse was true. In additional trials the fatty acids were mixed with the fat before adding salt solution, or mixed with salt solution before adding fat; the data obtained were similar to those given in table 3. In general, it appears that higher volatile fatty acids would be largely in the cheese fat rather than in the serum.

DISCUSSION

The cheesy flavor in the fat of aged cheddar cheese suggests that a change in this constituent may be important from the standpoint of flavor development in the cheese, and the relatively high acid number of the fat and the increase in the acid number during ripening indicate the importance of acid in this connection. Although acids produced from the

TABLE 3. *Distribution of fatty acids in butter fat and in 4 per cent sodium chloride solution*

About 0.2 ml. of a fatty acid was added to 20 ml. melted butter fat and 20 ml. 4 per cent NaCl solution. The mixture was shaken and held 7 days at 7.2°C. The fat and NaCl solution were separated, filtered and 10 ml. of each titrated.

Fatty acid added	Ml. 0.1N. alkali required to neut.		Percentage acid retained in	
	10 ml. fat	10 ml. NaCl sol.	Fat	NaCl sol.
Acetic	1.0*	26.6*	2	98
Propionic	2.5*	11.9*	14	86
Butyric	5.4*	7.0*	41	59
Caproic	9.8*	0.9*	91	9
Caprylic	9.1*	0.1*	99	1
Capric	6.2*	0.05*	> 99	< 1
Lauric	5.6*	0.05*	> 99	< 1
Myristic	2.0*	0.05*	> 99	< 1
Control	0.5	0.05	....	...

* Control subtracted from these values before calculating percentages.

non-fatty constituents of cheese might be dissolved by the fat, most of such acids apparently have a relatively low solubility in fat, and a more logical source of the acids present in the fat appears to be fat hydrolysis; this could yield various fatty acids that are readily soluble in fat. Undoubtedly, the volatile acids from fat have more effect on the flavor of cheese than the non-volatile. Because of its conspicuous odor, butyric acid would be expected to be prominent among the products of fat hydrolysis. This was not the case on the basis of the odor of cheese fat or of material distilled from cheese, but there remains the possibility of butyric acid being rapidly changed to other compunds through the action of organisms.

Hydrolysis of fat in cheddar cheese could be brought about by microorganisms or milk lipase. Although lipolytic organisms are not numerous in plates poured with cheese in the usual way, some factor involved in their growth may prevent development on plates. Since lipase is regularly present in milk its action would be expected, unless prevented by acid, salt, pasteurization of the milk, etc.

The relatively rapid increase in the acid number of fat in raw milk cheese during ripening, compared with that of fat in pasteurized milk cheese, may explain the differences in ripening qualities of the two types.

CONCLUSIONS

1. In aged cheddar cheese considerable flavor suggestive of ripened cheese was contained in the fat.

2. The acid numbers of fats from aged cheddar cheese were higher than those of fats from normal, sweet cream butter and approached those of fats from butter that was definitely rancid. They were low compared to values on fats from blue (roquefort type) cheese.

3. Acid numbers of fats from fresh (2 days) cheddar cheese were relatively high compared to those of fats from sweet cream butter and increased with ripening. The values for raw milk cheese were higher when the cheese was fresh and increased more rapidly than those for pasteurized milk cheese.

4. Volatile acidities of raw milk cheddar cheese were regularly higher than those of pasteurized milk cheese during the 200 days of ripening; the values for the former usually decreased slightly after 90 days while those for the latter continued to increase.

5. In general, the flavors of cheddar cheese from both raw and pasteurized milk were satisfactory after 200 days ripening. Raw milk cheese had considerable typical flavor, whereas pasteurized milk cheese usually was somewhat lacking in flavor.

6. When samples of aged cheddar cheese were distilled with steam and the distillates extracted with ether, oily residues were obtained which had "cheesy" aromas. The aromas suggested some of the higher volatile fatty acids, although certain of the residues apparently contained other aroma materials. Larger amounts of residue were obtained from raw milk cheese than from pasteurized milk cheese and the odors of the former were the more suggestive of aged cheese. Comparable residues were obtained from fat of aged cheddar cheese.

7. The solubility of fatty acids in butter fat, as would be expected, increased as the molecular weights of the acids increased, whereas in 4 per cent sodium chloride solution the reverse was true.

LITERATURE CITED

1. BREAZEALE, D. F., and E. W. BIRD.
1938. A study of methods for the determination of acidity in butter fat. Jour. Dairy Sci. 21:335-344.

2. CURRIE, J. N.
1914. Flavor of roquefort cheese. Jour. Agr. Res., 2:1-14.

3. LANE, C. B., and B. W. HAMMER
1935. Bacteriology of cheese. I. Effect of pasteurizing the milk on the nitrogenous decomposition in cheddar cheese. Iowa Agr. Exp. Sta., Res. Bul. 183.

4. LANE, C. B., and B. W. HAMMER
1938. Bacteriology of cheese. III. Some factors affecting the ripening of blue (roquefort type) cheese. Iowa Agr. Exp. Sta., Res. Bul. 237.

5. ORLA-JENSEN, S.
1904. Biologische Studien über den Käsereifungsprozess unter spezieller Berucksichtigung der flüchtigen Fettsäuren. Landw. Jahrb. Schweiz, 18:319-405.

6. SUZUKI, S. K., E. G. HASTINGS, and E. B. HART
1910. The production of volatile fatty acids and esters in cheddar cheese and their relation to the development of flavor. Wis. Agr. Exp. Sta., Res. Bul. 11.

7. WINDISCH, KARL
1901. Über die Veränderungen des Fettes beim Reifen der Käse. Arb. K. Gesundheitsamte 17:1-160. 1901. Abst. in Chem. Centbl. 72:128-130.

PRESSURE-AERATION EFFECTS ON THE DISSIMILATION OF GLUCOSE BY AEROBACTER INDOLOGENES

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The work of Brewer et al. (1938) has shown the marked effect of pressure on fermentation processes occurring in butter cultures. Aeration under pressures of 15 to 60 pounds per square inch induced a marked increase in the formation of diacetyl. In the following work the dissimilation of glucose by organisms of the genus Aerobacter has been studied under pressure to determine whether atmospheric oxygen under pressure may compete with hydrogen acceptors formed by the dissimilation and thus materially alter the normal course of fermentation especially with respect to the formation and reduction of acetylmethylcarbinol. The experimental work was conducted with the same equipment used by Brewer et al.

EXPERIMENTAL

Experiments were conducted at 30° C. in 500 ml. Erlenmeyer flasks provided with inlet and outlet tubes and containing 300 ml. of the following medium: 2.0 per cent glucose, 0.3 per cent $(NH_4)_2SO_4$ and 0.1 M diphosphate buffer. Since initiation of growth is slow under pressure the inoculum for each flask consisted of about 4 grams of cell paste obtained by centrifuging a 24-hour broth culture of *A. indologenes* (23B). One flask had 0.5 per cent acetic acid added to the glucose medium to permit observation of the effect of pressure-aeration on the conversion of acetic acid to neutral compounds (Reynolds and Werkman, 1937; Mickelson and Werkman, 1938). Air carried into the medium was washed free of carbon dioxide by passing through soda-lime towers. Gases were collected in a train placed outside the pressure tanks.

Hydrogen was determined gravimetrically by combustion over heated copper oxide. Carbon dioxide was collected in Bowen potash bulbs in a suitable drying train. Residual carbon dioxide was determined gravimetrically by acidifying to congro red and refluxing an aliquot part of the liquor in a stream of carbon dioxide-free air.

Acetylmethylcarbinol was determined by the iodoform method as applied by Langlykke and Peterson (1937). However, it was found that acetylmethylcarbinol was quantitatively recovered in six volumes by steam distillation. The reaction was carried out in an ice bath to avoid interference by ethyl alcohol which is present in the actylmethylcarbinol distillate.

2,3-Butylene glycol was determined according to Brockmann and Werkman (1933).

Ethyl alcohol was determined according to Stahly, Osburn and Werkman (1934) on the neutral volatile fraction. An aliquot part of the fermentation was made acid to congo red to prevent foaming and

TABLE 1. *Effect of aeration on the fermentation of glucose by Aerobacter indologenes*

Medium: 2.0 per cent glucose, 0.3 per cent $(NH_4)_2SO_4$, 0.1 M Phosphate buffer. Incubation: 30° C. for 1 week. Products per 100 mM glucose fermented.

Condition of aeration	CO_2	H_2	Acetic acid	Formic acid	Lactic acid	Acetyl-methyl-carbinol	2,3-Butylene glycol	Ethyl alcohol	Acetic acid fermented	Increase 2,3-butylene glycol	Acetyl-methyl-carbinol + 2,3-butylene glycol	Per cent C as acetyl-methyl-carbinol	Carbon recovery
A-anaerobic control	164.00	41.50	0.66	16.40	4.30	1.15	64.50	58.20			65.65	1.62	95.5
B-atmos. pressure	212.20	49.90	33.40	2.54	0	6.70	60.00	17.30			66.70	4.45	97.
C-45 lbs. per sq. in.	202.40	16.60	15.00	0.87	6.12	11.00	57.70	22.00			68.70	7.37	95.
D*-45 lbs. per sq. in.	207.00	0	81.40	2.54	0	15.60	66.00	9.62	31.20	15.30	81.60	9.42	92.2

* 88.2 mM acetic acid added per liter.

sugar breakdown and directly distilled to one-half volume. The distillate was then made alkaline to phenolphthalein and distilled again to one-half volume. The alcohol was determined on this distillate. A correction was applied for the acetylmethylcarbinol present in the sample.

The two residues of the above procedure were combined, acidified to congo red, and steam distilled to remove volatile acids. When acetylmethylcarbinol was present, the distillate was made alkaline to phenolphthalein, evaporated to a small volume, and steam distilled from the alkaline solution until six volumes were collected. This procedure removes acetylmethylcarbinol which interferes with the determination of formic acid. The residue was then acidified and redistilled. Formic acid was determined according to Auerbach and Zeglin (1922) and subtracted from the total acidity to obtain the acetic acid.

DISCUSSION

The results in table 1 indicate that aeration increases the yields of carbon dioxide and acetylmethylcarbinol but decreases the ethyl alcohol. The acetylmethylcarbinol plus butylene glycol is not affected by simple aeration or aeration under pressure for accompanying the increase in acetylmethylcarbinol there is a decrease in 2,3-butylene glycol. Acetic acid, which is reduced by *A. indologenes* (Reynolds, Jacobsson and Werkman, 1936) accompanied by an increase in four-carbon neutral compounds, undergoes similar reduction when the dissimilations are aerated under pressure. Apparently atmospheric oxygen under pressure is unable to compete with acetic acid as a hydrogen acceptor in this case and thus prevent its reduction to 2,3-butylene glycol.

Although the yield of gaseous hydrogen is considerable at atmospheric pressure a marked diminution occurs under pressure-aeration; the yield of ethyl alcohol is also reduced in the pressure-aerated fermentations. The carbon which ordinarily would be found as ethyl alcohol is recovered as carbon dioxide and acetic acid.

The variation of hydrogen and alcohol is of interest. Since the alcohol is formed by reduction, a decrease must be accompanied by an increase in some other reduced product or hydrogen. At atmospheric pressure (dissimilation B) the decrease in alcohol is accompanied by a considerable yield of gaseous hydrogen. Under the conditions the reduction of acetaldehyde to ethyl alcohol was prevented and yet the hydrogen thus not used, was not oxidized to water. When aerated under a pressure of three atmospheres (fermentation C) some of this excess hydrogen is oxidized and in fermentation D where acetic acid was added the yield of hydrogen is completely suppressed.

Kluyver and Donker (1925) showed that yeast and lactic acid bacteria form acetylmethylcarbinol from glucose when methylene blue or sulphur is added as a hydrogen acceptor. Presumably, the hydrogen acceptor prevents the reduction by yeast of intermediate acetaldehyde, which then condenses to form the carbinol. Oxygen in our aerated fermentations did not produce such an effect. On the contrary the decrease in ethyl alcohol is accompanied by increases in carbon dioxide and acetic acid. It is strange that products such as acetylmethylcarbinol and 2,3-butylene glycol, whose formation requires a reduction, should be formed in such constant yields in spite of a rather high oxygen tension. The results reported here have been repeatedly verified.

The increase in acetylmethylcarbinol in the aeration experiments occurs at the expense of 2,3-butylene glycol. Whether this is due to an inhibition of the reduction of acetylmethylcarbinol or to an oxidation of the glycol is not known. Walpole (1911) has shown that the formation of acetylmethylcarbinol from glucose by *A. aerogenes* is stimulated by the presence of oxygen and that similar conditions cause an oxidation of 2,3-butylene glycol to acetylmethylcarbinol. Werkman (1930) found 2,3-butylene glycol, when used as a sole source of carbon by species of Aerobacter, to be converted to acetylmethylcarbinol.

The fact that the reduction in yield of alcohol is not accompanied by an increase in acetylmethylcarbinol may be evidence against the aldehyde condensation conception of formation of acetylmethylcarbinol. There is no convincing evidence that Aerobacter forms this compound by a condensation of acetaldehyde.

CONCLUSIONS

Aeration and especially aeration under pressure causes increases in acetylmethylcarbinol but the yield of acetylmethylcarbinol plus 2,3-butylene glycol is equal to that of an anaerobic fermentation.

The yield of ethyl alcohol is diminished with a corresponding increase in carbon dioxide and acetic acid.

Added acetic acid is reduced in spite of the increased oxygen pressure.

REFERENCES

1. Auerback, F., and H. Zeglin
   1922. Beitrage Zur Kenntnis der Ameisensaüre. Z. physik Chemie 103: 161–177.
2. Brewer, C. R., M. B. Michaelian, C. H. Werkman, and B. W. Hammer
   1938. Effect of aeration under pressure on diacetyl production in butter cultures. Res. Bull. 233, Ia. State Coll. 42–56.
3. Brockmann, M. C., and C. H. Werkman
   1933. Determination of 2,3-butylene glycol in fermentations. Ind. Eng. Chem. Anal. Ed. 5: 206–207.
4. Friedemann, T. E., and J. B. Graesser
   1933. The determination of lactic acid. J. Biol. Chem. 100: 291–308.
5. Kluyver, A. J., and H. J. L. Donker
   1925. The formation of acetylmethylcarbinol and 2,3-butylene glycol in the fermentation of sugars by alcohol yeasts and true lactic acid bacteria. Koninklijke Akademie van Wetenschappen te Amsterdam. Proceedings 28: Pt. I 314–317.
6. Langlykke, A. F., and W. H. Peterson
   1937. Determination of acetylmethylcarbinol. Ind. Eng. Chem. Anal. Ed. 9: 163–166.
7. Mickelson, M. N., and C. H. Werkman
   1938. Influence of pH on the dissimilation of glucose by *Aerobacter indologenes*. J. Bact., 36: 67–76.
8. Reynolds, H., B. J. Jacobsson, and C. H. Werkman
   1937. The dissimilation of organic acids by *Aerobacter indologenes*. J. Bact. 34: 15–20.
9. Stahly, G. L., O. L. Osburn, and C. H. Werkman
   1934. Quantitative determination of acetone and ethyl, butyl and isopropyl alcohols in fermentation liquors. Analyst 59: 319–325.
10. Walpole, G. S.
    1911. The action of *Bacillus lactis aerogenes* on glucose and mannitol. Part II. Investigation of the 2,3-butenediol and aceteylmethylcarbinol formed; the effect of free oxygen on their production; the action of *B. lactis aerogenes* on glucose. Proc. Roy. Soc. (London) B. 83: 272–286.
11. Werkman, C. H.
    1930. An improved technique for the Voges-Proskauer test. J. Bact., 20: 121–125.

DECOMPOSITION AND GAS PRODUCTION OF CORNSTALKS UNDER ANAEROBIC CONDITIONS AT 28° TO 30° C.

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Enormous quantities of cornstalks, cereal straws, and other fibrous wastes are produced annually. Fermentation of these raw materials has been suggested as a possible preliminary treatment in the preparation of pulp. Utilizable gases, such as methane and carbon dioxide, can be produced also as by-products. The following investigation is concerned with the effect of preliminary fermentation on the constituents of cornstalks.

Acharya (1), employing soil infusion as inoculum, determined the rate of decomposition of the various plant constituents and studied some of the products formed when rice straw was fermented in glass jars or other tightly sealed containers from which air had been almost completely exhausted. He reported 30° to 35° C. and pH 7.5 to 7.8 as optimum conditions. Hemicelluloses and cellulose were readily decomposed but lignin was more resistant. The rates of decomposition were rather slow, the usual fermentation period being six months.

Tenney and Waksman (12), using garden soil extract as an inoculum, studied the decomposition of several materials, including cornstalks. Glazed jars were used and anaerobic conditions were obtained by covering the fermenting materials with water. Over relatively long periods of time (about 500 days), the pentosans (hemicelluloses) and cellulose were attacked to a considerable extent but lignin was more resistant.

Buswell and Boruff (3, 4, 5) studied the production of methane and carbon dioxide from several waste materials including cornstalks. They used the overflow liquor from a sewage disposal plant as an inoculum and developed a tank for this type of anaerobic fermentation. Wastes were fed into the tank daily and the residues remaining after fermentation were analyzed. The gas produced was measured and analyzed. They suggested that the residues might be used in the manufacture of pulp or paper. All the principal constituents of the stalks were broken down by this fermentation, cellulose showing the highest percentage loss.

In the work just discussed, either the rates of decomposition were studied over long periods, as exemplified by Acharya's and Waksman's reports, or only the final products of break-down at the termination of the experiments were ascertained, as shown in Buswell's studies. The long fermentation periods employed would not be feasible for production of residues for pulping because of the excessive break-down of cellulose and the high initial cost of the necessary equipment.

Information concerning the rate of gas production and break-down of cornstalks over short periods of fermentation is meager. To obtain such information, which is necessary in the development of a fermentation-pulping procedure, a series of anaerobic studies on cornstalks was made.

[1] This laboratory established at Ames, Iowa, by the Bureau of Chemistry and Soils, United States Department of Agriculture, in cooperation with the Iowa State College.

EXPERIMENTAL

The test materials were chopped cornstalks and cornstalk flour. The chopped cornstalks were prepared by means of a silage shredder and hand-picked pieces one-fourth to one inch in length (including leaves, pith, nodes, and outer shell) were selected. The cornstalk flour was prepared by wet-grinding the stalks in a rod mill and drying. Most of this flour passed a 200-mesh sieve.

The seed or inoculum was prepared from sewage sludge as previously described by the authors (7) and passed through a screen (circular opening 1 mm. in diameter) and all solids remaining on the screen were discarded. The ammonia-nitrogen content was determined and di-ammonium phosphate and ammonium carbonate were added to give a concentration of 500 to 800 p.p.m. of ammonia nitrogen.

The desired quantities of seed and test material were placed in two-quart mason jars fitted with three-hole rubber stoppers. A glass tube (12 mm.) was inserted through one of the holes in the stopper and extended to a point near the bottom of the jar. This tube was used for the removal of samples for pH determinations. A similar tube was used for the addition of chemicals when necessary. A 7-mm. glass tube, which was inserted into the other hole of the stopper to a point just beneath its bottom surface, served as an outlet for the gas produced. This gas tube was connected to a water seal in order to maintain anaerobic conditions. The gas volume was measured as previously described (7).

The fermentation mixture was prepared by placing 1000 cc. of seed into a mason jar and adding 25 grams of cornstalk material and 500 cc. of tap water. The stopper was then tightly inserted and secured by a mason jar screw cap (center portion cut out). The gas evolution tube was connected to a water seal and the mixture incubated at 28° to 30° C. Samples thus prepared were fermented for periods of 3, 5, 10, 20, and 30 days.

A daily record was made of the gas produced and the pH was frequently checked. The gas was analyzed for carbon dioxide, hydrogen, and methane by means of a modified Orsat apparatus (6).

At the end of the specified period the sample was removed and the total volume measured. A 200 cc. portion was removed for "sanitary" analysis, and the remainder evaporated to a dry state on a steam hot plate. The dried residue was ground to pass a 30-mesh screen and analyzed.

The "sanitary" analysis of the 200 cc. portion consisted of the following determinations: total solids, "volatile solids" (loss on ignition), ash, specific gravity, ammonia nitrogen, and Kjeldahl nitrogen according to the methods outlined in "Standard Methods for the Examination of Water and Sewage" (10).

The dried residue was analyzed for moisture, total solids, volatile solids, and ash; the pentosans were determined by the A. O. A. C. method (2); the cellulose by the Norman and Jenkins' method (8); and lignin (on unextracted material) by the Peterson, Walde, and Hixon modification of Bray's method (9).

The study may conveniently be divided into the following three phases:

1. Anaerobic decomposition of chopped and of ground cornstalks submerged in an active methane-producing seed.

2. The effect of screening and washing on the residues from anaerobic fermentation of chopped cornstalks.

3. Anaerobic decomposition of chopped and of ground cornstalks submerged in water.

RESULTS AND DISCUSSION

1. *Anaerobic decomposition of chopped and of ground cornstalks submerged in an active methane-producing seed.*

The cumulative amounts of gas produced from the cornstalk flour and the chopped cornstalk series are shown in Table I and in Figure 1. Gas was produced more rapidly from the cornstalk flour than from the chopped stalks; the respective amounts, per gram of volatile solids added,

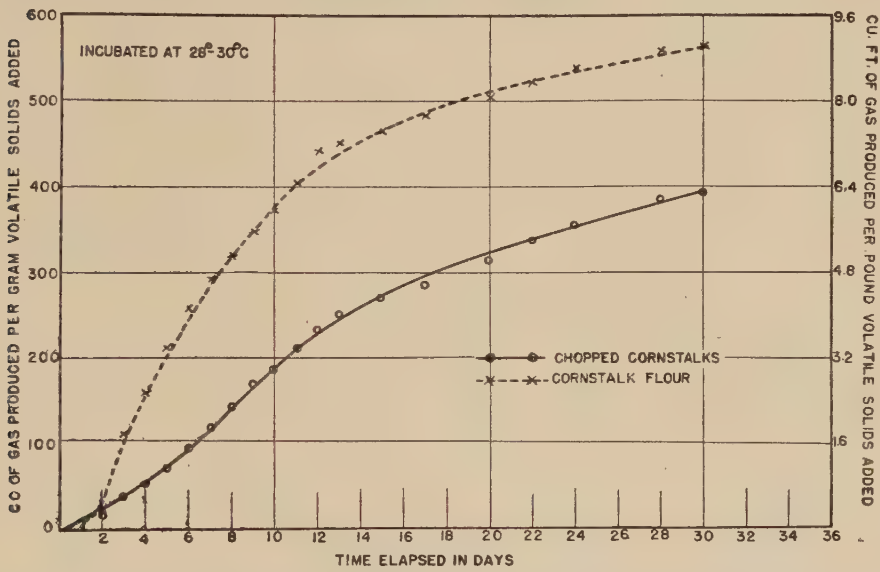

Figure 1. Gas Production from Cornstalks Submerged in an Active Methane Producing Seed at 28°-30° C.

were 115 and 41 cc. after 3 days, 213 and 74 cc. after 5 days, 374 and 188 cc. after 10 days, 507 and 316 cc. after 20 days, and 565 and 395 cc. after 30 days of fermentation. The average composition of the gas produced from the cornstalk flour was carbon dioxide 33.7 per cent, hydrogen 5.6 per cent, methane 58.9 per cent, and that from the chopped cornstalks, carbon dioxide 35.2 per cent, hydrogen 7.1 per cent, and methane 54.8 per cent.

Decomposition of the principal constituents is shown in Table II for the cornstalk flour and in Table III for the chopped cornstalks, and graphically in Figure 2. The percentage decomposition has been calculated on two different bases: (a) by attributing the loss entirely to the cornstalk material added and (b) by distributing the losses between the seed and the material added. The graphs were plotted from the latter figures because of the high lignin content of the seed and rather low lignin content of the stalks. Any experimental error attributed only to the stalks in this instance would be unduly magnified.

Table I. Gas Production from Cornstalks[1] at 28°-30° C.

Fermentation Period in Days	1	2	3	4	5	6	7	8	9	10	11	12	13	15	17	20	22	24	28	30
Cornstalk Flour[2]																				
cc. of Gas* per Gram Vol. Solids Added	8.0	20.3	114.5	161.5	213	260	294	322	348	374	407	443	452	467	484	507	524	540	560	565
pH	7.7	7.4	7.2	7.1	7.2	7.2	—	7.2	—	7.2	—	—	7.2	—	7.3	7.4	—	7.5	—	7.4
Chopped Cornstalks[3]																				
cc. of Gas* per Gram Vol. Solids Added	11.8	19.7	41.3	55.0	74.4	96.5	122	146	172	188	214	235	252	272	286	316	338	357	387	395
pH	7.6	7.5	7.5	7.5	7.5	7.5	—	7.6	—	7.5	—	—	7.5	—	7.5	7.5	—	7.6	—	7.6
Seed Control																				
Total Gas in cc.	90	90	90	90	90	90	90	90	135	180	180	180	180	225	270	315	360	445	445	490
pH	7.9	7.7	7.7	7.7	7.8	7.8	—	7.7	—	7.7	—	—	7.7	—	7.9	7.7	—	7.7	—	7.7

[1] Corrected to 760 mm. pressure and 60° F.
[2] Cornstalk flour mixture—average gas composition—CO_2 33.7%, H_2 5.6%, CH_4 58.9%.
[3] Chopped cornstalk mixture—average gas composition—CO_2 35.2%, H_2 7.1%, CH_4 54.8%.
* Cumulative.

			Materials Added			After Incubating				
			As Seed	As Cornstalk Flour	Total	Three Days	Five Days	Ten Days	Twenty Days	Thirty Days
Constituents* Present in Grams	Total solids		31.49	23.66	55.15	51.87	48.88	44.50	41.74	39.93
	Volatile solids		21.26	22.42	43.68	40.70	37.19	33.29	30.40	28.86
	Pentosans		3.26	7.66	10.92	9.00	6.29	5.95	5.07	4.56
	Crude cellulose (ash free)		5.69	11.30	16.99	16.43	10.40	7.71	6.45	5.66
	Pentosans in the cellulose		1.41	3.37	4.78	4.96	3.25	2.36	1.71	1.21
	Cellulose (corrected)		4.28	7.93	12.21	11.47	7.15	5.35	4.98	4.45
	Lignin		9.89	4.53	14.42	14.34	14.20	14.24	14.01	13.49
Percentage Decomposition of Constituents	Total solids	α*				13.85	26.45	44.98	56.60	64.30
		$\beta\delta$				13.37	25.90	43.35	51.35	55.60
	Volatile solids	α*				13.29	28.97	46.26	59.29	66.05
		$\beta\delta$				12.57	23.50	45.20	54.60	59.90
	Pentosans	α*				25.00	60.40	64.90	76.25	83.00
		$\beta\delta$				23.20	57.55	58.95	71.95	78.80
	Crude cellulose (ash free)	α*				4.86	58.20	82.05	91.00	100.00
		$\beta\delta$				4.61	57.60	80.80	90.90	97.20
	Pentosans in the cellulose	α*				+ 5.34	45.45	71.75	91.00	106.00
		$\beta\delta$				+ 4.74	45.45	70.55	89.50	104.00
	Cellulose (corrected)	α*				9.34	63.85	86.50	91.00	97.80
		$\beta\delta$				8.57	62.90	85.20	88.60	94.45
	Lignin	α*				1.76	4.85	3.97	9.06	20.53
		$\beta\delta$				+ 2.20	+ 1.54	+ 7.94	+ 3.09	8.15
Gas Produced in cc. 760 mm. and 60° F.	Total					2565	4775	8385	11380	12655
	Per gram cornstalk flour added					103	191	335	455	506
	Per gram vol. solids in flour added					115	213	374	507	563
	Per gram vol. solids lost	α*				850	737	808	857	853
		$\beta\delta$				910	903	826	927	940

α* On assumption that total loss may be attributed to cornstalk flour added.

$\beta\delta$ On assumption that loss was distributed between seed and flour added and that decomposition of seed in mixture was the same as in seed control. (See table VI.)

Initial volume 1500 cc. Initial Organic N_2 508 p.p.m.

Initial NH_3 content 782 p.p.m. Initial reaction = pH 7.8

$$\text{Ratio} \frac{\text{Vol. solids in flour}}{\text{Vol. solids in seed}} = 1.058$$

+ (plus)—indicates an increase—due to sampling difficulties and in the case of lignin probably due to formation of nitrogen complexes.

* Kjeldahl nitrogen determinations were run on all of the samples, but since no extensive accumulation or decrease of nitrogen was noted, these value were not included in this table.

Table III. Decomposition of and Gas Production from Chopped Cornstalks at 28°-30° C.

			Materials Added			After Incubating				
			As Seed	As Chopped Cornstalks	Total	Three Days	Five Days	Ten Days	Twenty Days	Thirty Days
Constituents* Present in Grams	Total solids		31.49	24.15	55.64	51.35	49.60	46.50	43.80	41.60
	Volatile solids		21.26	22.35	43.61	40.80	38.42	35.95	33.15	30.88
	Pentosans		3.26	6.50	9.76	9.41	8.62	7.49	6.51	5.64
	Crude cellulose (ash free)		5.69	11.37	17.06	15.04	13.11	12.63	11.52	10.71
	Pentosans in the cellulose		1.41	2.94	4.35	2.96	3.35	3.11	2.01	2.10
	Cellulose (corrected)		4.28	8.43	12.71	12.08	9.76	9.52	9.51	8.61
	Lignin		9.89	4.49	14.38	13.97	13.61	13.20	12.84	12.85
Percentage Decomposition of Constituents	Total solids	α*				17.75	24.90	37.85	49.05	58.15
		βδ				16.95	24.42	36.18	43.05	49.55
	Volatile solids	α*				12.58	23.22	34.35	46.80	56.95
		βδ				11.88	22.32	33.21	42.25	50.90
	Pentosans	α*				5.38	17.55	34.90	50.00	63.35
		βδ				3.23	14.30	29.55	44.70	58.45
	Crude cellulose (ash free)	α*				19.15	34.75	39.00	48.70	55.90
		βδ				17.42	34.04	37.70	46.80	53.00
	Pentosans in the cellulose	α*				47.20	34.00	42.60	79.45	76.55
		βδ				47.20	34.00	41.75	77.20	74.50
	Cellulose (corrected)	α*				7.47	35.00	37.82	37.90	48.60
		βδ				6.76	34.05	36.62	35.70	45.45
	Lignin	α*				9.13	17.14	26.20	34.25	34.08
		βδ				5.12	10.91	14.25	22.05	21.60
Gas	Total					965	1745	4385	7385	9230
Produced in cc. 760 mm. and 60° F.	Per grams chopped cornstalks added					40.0	72.2	181.3	305.5	382
	Per gram vol. solids in stalks added					43.2	78.1	196.0	330.0	414
	Per gram vol. solids lost	α*				344	336	572	705	725
		βδ				364	350	590	782	812

α* On assumption that total loss may be attributed to chopped stalks added.

βδ On assumption that loss was distributed between seed and chopped stalks added and that decomposition of seed in mixture was the same as in seed control. (See table VI.)

Initial volume 1500 cc. Initial Organic N_2 409 p.p.m.

Initial NH_3 content 828 p.p.m. Initial reaction = pH 7.8

$$\text{Ratio} \frac{\text{Vol. solids in stalks}}{\text{Vol. solids in seed}} = 1.053$$

* Kjeldahl nitrogen determinations were run on all of the samples, but since no extensive accumulation or decrease of nitrogen was noted, these values were not included in this table.

The cellulose in the cornstalk flour was decomposed more rapidly than that in the chopped stalks. Respective amounts of cellulose decomposition in the flour and chopped stalks were 9 and 7 per cent in 3 days, 63 and 43 per cent in 5 days, 85 and 37 per cent in 10 days, 89 and 36 per cent in 20 days, and 94 and 45 per cent in 30 days.

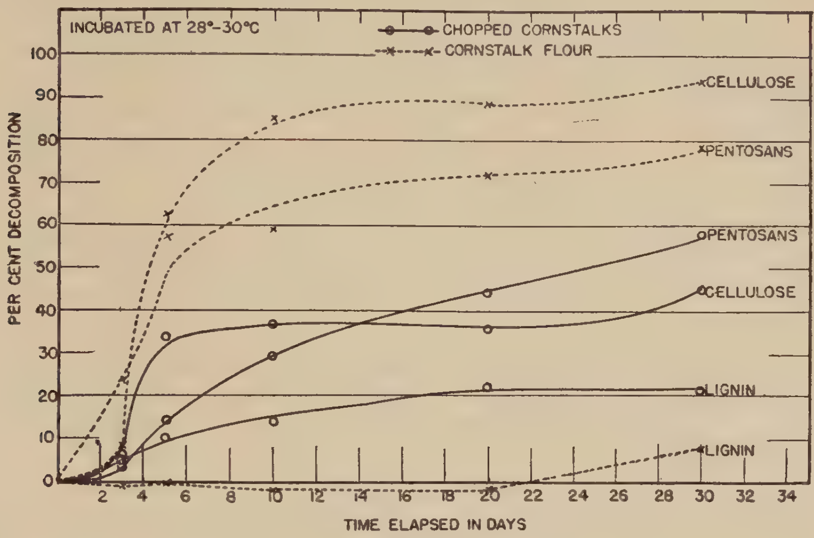

Figure 2. Break-down of Cornstalk Flour and Chopped Cornstalks Submerged in an Active Methane Producing Seed at 28°-30° C.

In Figure 3 the decomposition of cellulose and of the pentosans associated with the cellulose is shown. In the cornstalk flour the decomposition of the pentosans associated with the cellulose lagged behind that of the cellulose for the first 10 days of fermentation, but, thereafter, the remaining cellulose was resistant to decomposition, whereas the pentosans associated with cellulose continued to be decomposed. In the case of the chopped cornstalks the pentosans associated with the cellulose were decomposed more rapidly than the cellulose itself, especially in the later stages of fermentation.

The break-down of the pentosans in the cornstalk flour and in the chopped stalks is shown in Tables II and III and in Figure 2. The pentosans in the cornstalk flour were fermented more rapidly than those in the chopped stalks, the respective amounts of decomposition being 23 and 3 per cent in 3 days, 58 and 14 per cent in 5 days, 59 and 30 per cent in 10 days, 72 and 45 per cent in 20 days, and 79 and 59 per cent in 30 days. In the cornstalk flour series, with the exception of the first 3 days, there was a greater percentage loss of cellulose than of pentosans throughout the 30-day fermentation period. In the chopped cornstalk series however the percentage loss of cellulose was greater than that of the pentosans during the first 14 days, after which time the pentosan loss was greater. However, the total break-down of both cellulose and pentosans was much less in the chopped cornstalks than in the cornstalk flour.

The decomposition of lignin is also shown in Tables II and III and

Figure 2. In the cornstalk flour series there was no decomposition of the lignin during the first 22 days, after which time there was a gradual loss which totaled 8 per cent by the thirtieth day. In the chopped stalk series, however, lignin decomposed during the entire period of fermentation and showed a total loss of 22 per cent by the thirtieth day. This break-down of lignin is in contrast to the break-down of the pentosans and cellulose,

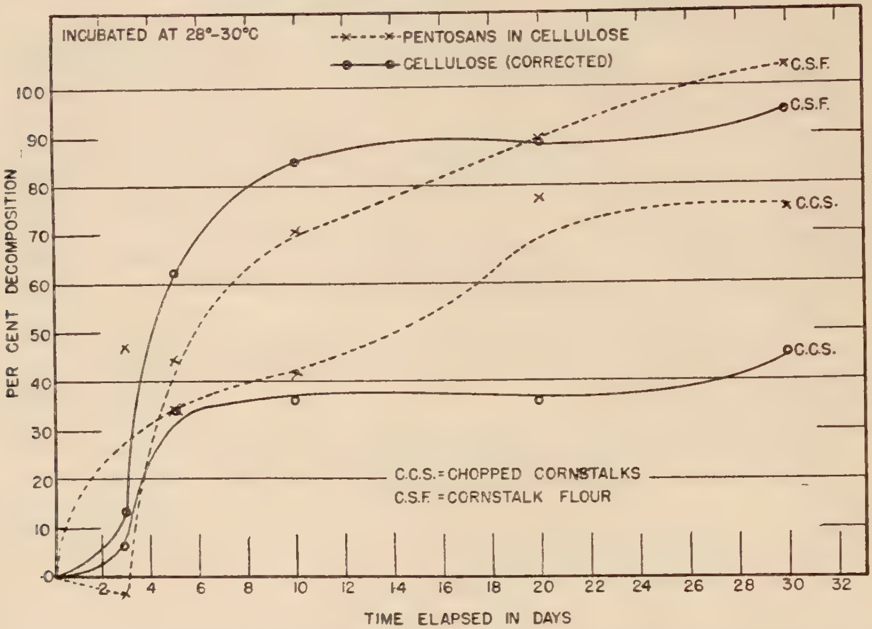

Figure 3. Decomposition of Cellulose and Pentosans Associated with Cellulose in Cornstalks submerged in an Active Methane Producing Seed at 28°-30° C.

which disappeared more rapidly in the cornstalk flour. The reason for this behavior has not been ascertained.

Boruff and Buswell (5) have calculated the theoretical gas yields obtainable from various plant constituents and were able to confirm the results experimentally. Using their theoretical values, a series of calculations, based on the loss of pentosans and cellulose (Tables II and III), was made and is presented in Table IV. In the cornstalk flour series, the ratio of gas produced to the theoretical value calculated from the pentosans and cellulose lost was 1.09: 1.0 for the 3-day fermentation period, 0.59: 1.0 for the 5-day, 0.85: 1.0 for the 10-day, 1.05: 1.0 for the 20-day, and 1.07: 1.0 for the 30-day fermentation period.

Although the loss of pentosans and cellulose is great enough to account for most of the gas produced during the 3-day period, the high recovery may be due to gas production from constituents other than pentosans and cellulose and that gas production from the two latter lags behind initial breakdown owing to the production of intermediates.

In the 5-day period the quantities of cellulose and pentosans broken down were far in excess of those accounted for by the gas produced. This

indicates that these two constituents decomposed faster than they were converted into gas. In the 10-day period their conversion to gas still lagged behind, but to a lesser degree.

In the 20-, and 30-day periods all the pentosans and cellulose broken down were gasified, thus accounting for practically all of the gas produced. The small amount of additional gas is attributed to the decomposition of volatile matter other than cellulose and pentosans.

In view of these data it is doubtful whether the volume of gas produced should be used as a direct measure of the break-down of cellulose and pentoans, except possibly at the beginning and completion of the fermentation.

In the chopped cornstalk series the ratio of gas produced to the theoretical value calculated from the pentosans and cellulose lost was 1.17: 1.0 for the 3-day fermentation period, 0.51: 1.0 for the 5-day, 0.96: 1.0 for the 10-day, 1.34: 1.0 for the 20- and 30-day fermentation periods.

Assuming that all the pentosans and cellulose which disappeared were converted into gas, such gasification would account for 83 per cent of the gas produced during the first three days of digestion, the remaining 17 per cent being attributed to other decomposable materials. After the 5-day period, however, the volume of gas produced is not adequate to account for the decrease in pentosan and cellulose content. This indicates that these two constituents were decomposed faster than they were being converted into gas. In the 10-day period the gas produced accounted for all but 4 per cent of the cellulose and pentosans decomposed. In the 20- and 30-day periods the pentosans and cellulose decomposed accounted for only 66 per cent of the gas produced. Volatile matter other than cellulose and pentosans produced the remaining 34 per cent.

It is noted (Table IV) that in the cornstalk flour series greater amounts of pentosans and cellulose were decomposed than in the chopped cornstalk series. This is attributed to difference in particle size, as pointed out in a previous article (7).

It will also be noted (Table IV) that most of the gas produced in the cornstalk flour series is accounted for by the pentosan and cellulose decomposition whereas the amount of gas produced in the chopped cornstalk series is greater than that resulting from such decomposition. The reason for this behavior is not known. It is suggested that the difference in the size of the particles may affect not only the rate but also the nature of the decomposition.

The results of the decomposition in the seed controls are presented in Table VI. Very little gas was produced and the decomposition of the various constituents was very slow.

Although no actual experimental work was done, the fermentation of cornstalk flour, using an active seed, may have possibilities of being useful for composting.

2. *The effect of screening and washing on the residues from the anaerobic fermentation of chopped cornstalks.*

In this study a series of samples of chopped cornstalks was prepared and incubated at 28° to 30° C. at the same time and in the same manner as the chopped cornstalk series described in the previous section. This series was an exact duplicate of the previous series, the only difference being that in the first series the entire sample, seed and residue, was

Table IV. A Comparison of the Total Gas Produced and of the Amounts Accounted for (by calculation) from the Cellulose and the Pentosans Decomposed

Time	Cornstalk Flour*					Chopped Cornstalks*				
	Loss in Grams		Gas in cc.		Ratio Gas as Measured : to	Loss in Grams		Gas in cc.		Ratio Gas as Measured : to
	Pentosans	Cellulose	As Measured	Theoretical Yield Calculated from Pentosans and Cellulose Decomposed	Theoretical Yield Calculated from Pentosans and Cellulose Decomposed	Pentosans	Cellulose	As Measured	Theoretical Yield Calculated from Pentosans and Cellulose Decomposed	Theoretical Yield Calculated from Pentosans and Cellulose Decomposed
3 days	1.92	0.74	2565	2346	1.093:1.000	0.35	0.63	965	821	1.175:1.000
5 days	4.63	5.06	4775	8136	0.587:1.000	1.14	2.95	1745	3418	0.511:1.000
10 days	4.97	6.86	8385	9918	0.845:1.000	2.27	3.19	4385	4578	0.957:1.000
20 days	5.85	7.23	11380	10815	1.052:1.000	3.25	3.20	7385	5519	1.338:1.000
30 days	6.36	7.76	12655	11848	1.068:1.000	4.12	4.10	9230	6905	1.336:1.000

* 25 grams of material.

evaporated to dryness and analyzed, whereas in this series the seed was screened off (using a 1-mm. round-hole screen) from the chopped stalks and the stalk material washed three times with a total of one liter of distilled water. The washed stalk matrial was then dried on a steam hot plate, ground to 30 mesh, and analyzed.

The percentage losses of the various constituents in the stalk-seed mixture and in the washed and screened stalk material is shown in Tables III and V respectively, and graphically in Figure 4. It should be pointed out that in the mixture the losses recorded are actual biological decompositions, since the entire mass was analyzed, while in the screened and

Table V. Effect of Screening and Washing the Chopped Cornstalk Residue*

		Initial Chopped Cornstalks	After Incubating Three Days	Five Days	Ten Days	Twenty Days	Thirty Days
Constituents Present in Grams	Total solids	24.15	19.84	16.80	14.90	12.47	10.54
	Volatile solids	22.35	19.24	16.50	14.63	12.11	10.18
	Pentosans	6.50	5.88	5.04	4.43	3.74	2.92
	Cellulose (corrected)	8.43	7.82	6.94	6.30	4.39	3.68
	Lignin	4.49	4.10	3.75	3.53	3.19	2.92
Percentage Decrease in Constituents	Total solids		22.00	30.45	38.30	48.75	56.40
	Volatile solids		13.93	26.20	34.60	45.80	54.45
	Pentosans		9.53	22.45	31.80	42.50	55.10
	Cellulose (corrected)		7.22	17.65	25.25	47.90	56.35
	Lignin		8.70	16.45	21.35	28.90	34.95

* Fermented at 28°-30° C.

washed stalk material part of the losses are not true decompositions but are due to the method of preparing the sample. In this study a procedure was followed that might be applicable in the fermentation of cornstalks for pulp production.

During the first 17 days of fermentation the cellulose loss in the stalk-seed mixture was greater than that in the screened and washed series after which time it was greater in the latter. It was expected that fine particles of cellulose would be liberated from the chopped cornstalks and accumulate in the mixture and subsequently be lost in the screened and washed samples. This anticipated mechanical loss did not become manifest until the later stages of the digestion.

During the first 16 days the pentosans disappeared somewhat more rapidly from the screened and washed samples than from the seed-mixture samples, after which time there was a somewhat greater loss in the latter samples.

Throughout the fermentation period (30 days) the lignin losses in the screened and washed samples were greater than in the seed-mixture samples. Lignin is not as readily decomposed by micro-organisms as are

Table VI. Decomposition and Gas Production of Seed Control at 28°-30° C.

		Seed at Start	After Incubating Three Days*	Five Days*	Ten Days	Twenty Days*	Thirty Days
Constituents Present in Grams	Total solids	31.49	31.40	31.35	31.09	30.25	29.43
	Volatile solids	21.26	21.10	21.05	21.03	20.25	19.90
	Pentosans	3.26	3.12	3.05	2.91	2.92	2.94
	Cellulose	4.28	4.22	4.20	4.18	4.09	4.01
	Lignin	9.89	9.80	9.69	9.44	9.43	9.42
Percentage Decrease in Constituents	Total solids		0.28	0.45	1.27	3.94	6.54
	Volatile solids		0.75	0.99	1.16	4.75	6.40
	Pentosans		4.30	6.44	10.73	10.41	9.82
	Cellulose		1.40	1.87	2.34	4.44	6.30
	Lignin		1.80	2.91	5.41	5.52	5.61
Gas Produced in cc. at 760 mm. and 60° F.	Total		90	90	180	315	360
	Gas per gram volatile solids lost		563	428	750	312	265

* The initial, 10-day and 30-day values were plotted and the values for the 3-, 5-, and 20-day periods determined from the graph.

the other cornstalk constituents. In the process of fermentation of the cellulose and pentosans, lignin particles are probably released and accumulate in the mixture and are subsequently lost in the process of screening and washing the fermented stalks. This fact must be kept in mind in studies on the fermentation of lignin by micro-organisms to prevent drawing erroneous conclusions.

The losses of the principal constituents of the fermented cornstalks by screening and washing are briefly summarized as follows:

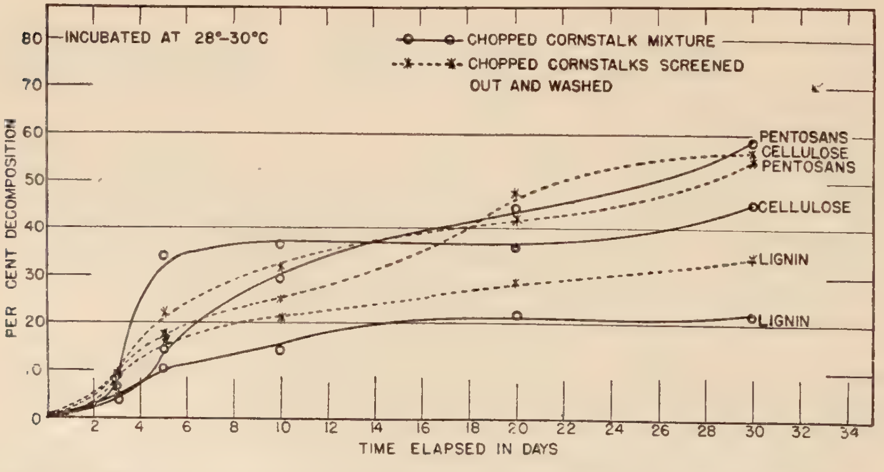

Figure 4. Effect of Screening and Washing on the Chopped Cornstalk Residue after Fermenting with a Prepared Seed.

In the fermentation of the cellulose and the pentosan, lignin is probably separated from the stalks and accumulates as small particles in the liquid.

Screening and washing removes this lignin thus giving a loss that is not entirely caused by microbial decomposition.

During the first 16 days of fermentation the pentosans were removed from the stalks more rapidly than they were being decomposed into gas and consequently accumulated in the liquid portion of the batch. Screening and washing removed a large portion of the accumulated pentosans. During the remaining 14 days of fermentation the pentosans were being decomposed and converted into gas at about the same rate.

During the first 16 days of fermentation the cellulose was gasified as rapidly as it was decomposed. During the next 14 days the cellulose was decomposed more rapidly than it was being gasified, thus accumulating in the liquid portion of the batch. Screening and washing removed a large portion of the accumulated cellulose, thus giving a loss that was not entirely due to microbial action.

Pulping the residue from a fermentation lasting longer than 2 weeks would not be feasible because of the large cellulose losses resulting from screening and washing.

3. *Anaerobic decomposition of chopped and of ground cornstalks submerged in water.*

Some reports have appeared in the literature on the production of pulp from cornstalks by fermentation and by a combination of fermentation and chemical treatment, using the microbial flora present on the stalks. Sweeney and Arnold (11) reported the production of a "superior pulp" and a saving in chemicals by the use of a fermentation and chemical treatment. The fermentation was obtained by means of the flora present on the cornstalks.

Acharya (1) showed that, by the addition of nitrogen salts to rice straw, the organisms originally present on the straw were capable of fermenting this material with the production of gas, but that the development of acid conditions prevented extensive fermentation.

In the present study a series of samples of cornstalk flour and chopped cornstalks was prepared as follows: 25-gram portions of the cornstalk material and 1500 cc. of tap water were placed in two-quart mason jars, and the jars were closed, sealed, and connected to water seals as in the previous studies. The samples were incubated at 28° to 30° C. for 5-, 10-, and 30-day periods.

Duplicate samples were prepared and used to measure, daily, the volumes of gas produced and also for pH determinations. These results are shown in Table VII. Very little gas was produced (360 cc. from the cornstalk flour and 175 cc. from the chopped stalks after 30 days of incubation). The initial pH, 6.2 to 6.3 in both series, decreased during the fermentation period, reaching pH 4.6 after 30 days.

The decomposition of the various constituents is shown in Table VIII for the cornstalk flour and in Table IX for the chopped cornstalks, and graphically in Figure 5. The amount of break-down of the various constituents was rather small in both the flour and stalks.

The amount of decomposition of cellulose was much higher in the

Table VII. Gas Production from Cornstalks Submerged in Water* at 28°-30° C.

Fermentation Period in Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	17	20	27	30
	Cornstalk Flour																	
Total Gas in cc.	90	180	360	360	360	360	360	360	360	360	360	360	360	360	360	360	360	360
pH	6.3	6.2	5.7	5.6	5.1	5.7	5.2	5.7	5.3	5.2	5.2	5.2	5.0	5.0	4.6	4.6	4.6	4.6
	Chopped Cornstalks																	
Total Gas in cc.	135	135	135	135	135	135	135	135	135	135	135	135	135	135	135	135	175	175
pH	6.2	6.2	6.4	5.7	5.4	5.7	5.4	5.7	5.5	5.6	5.7	5.4	5.2	5.2	4.7	4.7	4.6	4.6

* Corrected to 760 mm. pressure and 60° F.

Table VIII. Decomposition of Cornstalk Flour Submerged in Water 28°-30° C.

		Cornstalk Flour Used	After Fermenting Five Days	Ten Days	Thirty Days
Constituents Present in Grams	Total solids	23.66	23.31	23.56	22.19
	Volatile solids	21.70	21.45	21.66	20.52
	Pentosans	7.66	6.86	6.55	5.57
	Cellulose	7.93	7.62	7.67	7.44
	Lignin	4.53	4.63	4.85	4.73
Percentage Decrease in Constituents	Total solids		1.48	.42	6.21
	Volatile solids		1.15	.02	5.43
	Pentosans		10.43	14.48	27.28
	Cellulose		3.91	3.28	6.18
	Lignin		+ 2.21	+ 7.06	+ 4.41
Gas Produced in cc. at 760 mm. and 60° F.	Total		360	360	360
	Per gram cornstalk flour added		15.2	15.2	15.2
	Per gram volatile solids in cornstalk flour added		16.6	16.6	16.6
	Per gram volatile solids lost		1438.0	——	305.0

+ Plus indicates increases—probably due to formation of nitrogen complexes.

chopped cornstalks than in the flour although the total break-down in the former was only 22 per cent in 30 days of fermentation.

The pentosan decomposition was a little higher in the chopped stalks than in the flour during the first 13 days of incubation; after this time it

Table IX. Decomposition of Chopped Cornstalks Submerged in Water 28°-30° C.

		Chopped Cornstalks Used	After Fermenting Five Days	Ten Days	Thirty Days
Constituents Present in Grams	Total solids	24.14	22.27	22.39	20.98
	Volatile solids	21.32	19.88	20.10	18.57
	Pentosans	6.50	5.57	5.83	5.36
	Cellulose	8.43	7.65	7.59	6.51
	Lignin	4.49	4.73	4.42	4.51
Percentage Decrease in Constituents	Total solids		7.75	7.25	13.10
	Volatile solids		6.75	5.73	12.90
	Pentosans		14.32	10.31	17.53
	Cellulose		9.25	9.98	22.78
	Lignin		+ 5.35	0.16	+ 0.45
Gas Produced in cc. at 760 mm. and 60° F.	Total		135	135	175
	Per gram chopped corn-stalks added		5.6	5.6	7.3
	Per gram volatile solids in cornstalks added		6.3	6.3	8.2
	Per gram volatile solids lost		93.7	111.0	63.7

+ Plus values indicate increases—probably due to formation of nitrogen complexes.

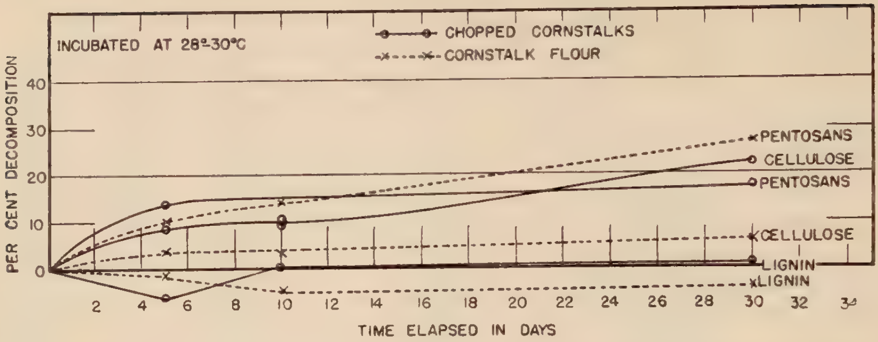

Figure 5. Break-down of Cornstalk Flour and of Chopped Cornstalks Submerged in Water at 28°-30° C.

was higher in the flour, showing a loss of 27 per cent after 30 days of fermentation.

No lignin loss was noted either in the chopped cornstalks or in the cornstalk flour. Slight increases in lignin were noted at times but these differences were within the limits of experimental error. In some cases, however, there were increases of 5 per cent or more. In fermentations such as studied here lignin-nitrogen complexes are frequently formed which would be analyzed as lignin (unpublished data). Similar lignin increases have been reported in the literature (11).

In this series of experiments the decomposition of the cellulose was somewhat greater in the chopped cornstalks than in the cornstalk flour. In the fermentations of the cornstalks submerged in a prepared seed, previously discussed, the losses were considerably greater in the cornstalk flour than in the chopped stalks. This result would be expected with the finer ground cornstalk flour. The behavior in the cornstalk series submerged in water might have been due to the manner of preparing the

Table X. The Effect of Filtering off and of Washing the Cornstalk Flour.*

		Cornstalk Flour Used	After Fermenting Five Days	Ten Days	Thirty Days
Constituents Present in Grams	Total solids	23.66	21.67	21.30	19.75
	Volatile solids	21.70	21.03	20.65	19.20
	Pentosans	7.66	6.38	6.17	5.43
	Cellulose	7.93	7.45	7.72	7.44
	Lignin	4.53	4.63	4.78	4.48
Percentage Decrease in Constituents	Total Solids		8.41	9.97	16.51
	Volatile solids		3.09	4.84	11.52
	Pentosans		16.70	19.45	29.10
	Cellulose		6.05	2.63	6.18
	Lignin		+ 2.20	+ 5.57	1.10

* Submerged in water and fermented at 28°-30°C.

+ Plus values indicate increases—probably due to formation of nitrogen complexes.

Table XI. Effect of Screening and Washing the Chopped Cornstalks.*

		Chopped Cornstalks Used	After Fermenting Five Days	After Fermenting Ten Days	After Fermenting Thirty Days
Constituents Present in Grams	Total solids	24.14	18.16	17.81	15.86
	Volatile solids	21.32	17.37	17.16	15.28
	Pentosans	6.50	5.26	5.12	4.66
	Cellulose	8.43	7.09	7.04	6.22
	Lignin	4.49	4.03	3.78	3.72
Precentage Decrease in Constituents	Total solids		24.77	26.22	34.30
	Volatile solids		18.54	19.54	28.32
	Pentosans		19.08	21.25	28.30
	Cellulose		15.88	16.48	26.20
	Lignin		10.26	15.82	17.17

* Submerged in water and fermented at 28°-30° C.

cornstalk flour and the chopped cornstalks. However, further investigation is needed for ascertaining the reasons for this behavior.

The effect on the losses of the various constituents in the cornstalk flour resulting from filtering off the liquid and washing (filtering and washing done at the end of fermentation period) is shown in Table X and graphically in Figure 6. Slightly larger losses of pentosans and lignin, due to the filtering and washing, are noted; they are slight and of no real significance. Little change in the cellulose losses due to the filtering and washing were noted. The increased losses of cornstalk flour resulting from filtering and washing were probably due to true solution of a portion of the constituents, or to a reduction to colloidal size permitting a passage through the filter paper, or both.

The effect on the losses of the various constituents in the chopped cornstalks resulting from the screening and washing is shown in Table XI and graphically in Figure 7. Increased losses in pentosans, cellulose, and lignin due to the screening and washing are indicated. These losses were probably caused by decrease in size of some of the chopped cornstalk particles permitting them to pass through the screen and into the liquid portion.

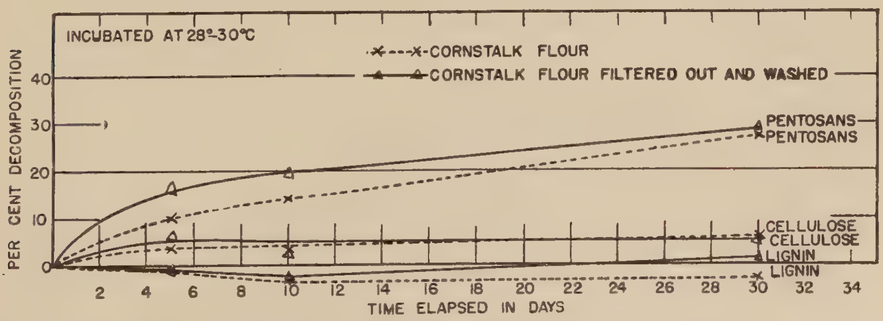

Figure 6. Effect of Filtering and Washing on the Cornstalk Flour Residue after Submerging in Water at 28°-30° C.

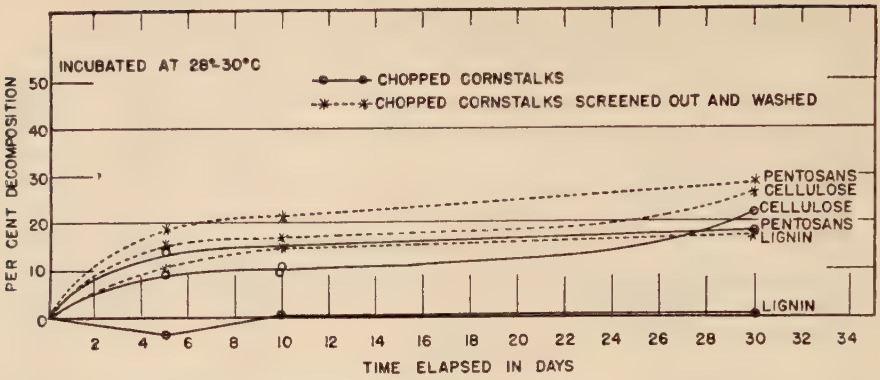

Figure 7. Effect of Screening and Washing on the Chopped Cornstalk Residue after Fermenting Without a Prepared Seed at 28°-30° C.

Submergence of cornstalks in water up to a period of 30 days at room temperature did not disclose any appreciable losses in lignin and pentosans. The decrease in chemicals required for pulping cornstalks submerged in water for various lengths of time (as has been reported in the literature) is, therefore, probably due to physical rather than to biological action.

SUMMARY AND CONCLUSIONS

1. In the anaerobic decomposition at 28° to 30° C. cornstalk flour was fermented more rapidly than chopped cornstalks, producing 43 per cent more gas in the 30-day period. This gas consisted of 34 to 35 per cent carbon dioxide and 55 to 59 per cent methane. The methane could be used for the production of heat or power and the carbon dioxide could be recovered for use in the manufacture of dry ice or other products.

2. In the cornstalk flour series approximately 95 per cent of the gas produced could be accounted for by the cellulose and pentosans decomposed for the 3-, 20-, and 30-day periods. For the same periods in the chopped cornstalk series only 75 per cent of the gas produced could be accounted for by the cellulose and pentosans decomposed.

3. For the 5- and 10-day periods, in both the cornstalk flour series and chopped cornstalk series, more cellulose and pentosans were decomposed than could be accounted for by the gas produced. This indicates that the rate of gas production cannot be taken as a criterion of the rate of break-down of the individual constituents of cornstalks during the earlier stages of decomposition.

4. In the cornstalk flour series the cellulose and pentosan losses were considerably greater than in the chopped cornstalk series. In the chopped cornstalk series the lignin loss was greater than in the cornstalk flour series.

5. By screening and washing the chopped cornstalk residue after fermentation periods of 3, 5, and 10 days, the pentosan losses were increased. Cellulose losses, however, were not increased. For the 20- and 30-day periods the pentosan losses were not increased by screening and

washing while the cellulose losses were increased. For all periods the lignin losses were increased by screening and washing.

6. The incubation of cornstalk flour and chopped cornstalks, submerged in water, showed small losses of the principal constituents. This procedure offers very little promise for use in pulp manufacture.

7. The fermentation of chopped cornstalks, using an active seed, shows some promise of being adaptable to the manufacture of pulp.

LITERATURE CITED

1. ACHARYA, C. N.
   (a) 1935. Studies on the Anaerobic Decomposition of Plant Materials. I. The Anaerobic Decomposition of Rice Straw. Biochem. J. **29**: 528.
   (b) 1935. Studies on the Anaerobic Decomposition of Plant Materials. II. Some Factors Influencing the Anaerobic Decomposition of Rice Straw. Biochem. J. **29**: 953.
   (c) 1935. Studies on the Anaerobic Decomposition of Plant Materials. III. Comparison of the Course of Decomposition of Rice Straw under Anaerobic, Aerobic, and Partially Aerobic Conditions. Biochem. J. **29**: 1116.
   (d) 1935. Studies on the Anaerobic Decomposition of Plant Materials. IV. The Decomposition of Plant Substances of Varying Compositions. Biochem. J. **29**: 1459.

2. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
   Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, Fourth Edition, Menasha, Wis. George Banta Publishing Company.

3. BUSWELL, A. M.
   1930. Production of Fuel Gas by Anaerobic Fermentation. Ind. Eng. Chem. **22**: 1168.

4. BUSWELL, A. M., and C. S. BORUFF.
   1933. Mechanical Equipment for Continuous Fermentation of Fibrous Materials. Ind. Eng. Chem. **25**: 147.

5. BORUFF, C. S., and A. M. BUSWELL.
   1930. Fermentation Products from Cornstalks. Ind. Eng. Chem. **22**: 931.

6. MATUSYAK, M. P.
   1934. Unitized Gas-Analysis Apparatus. Ind. Eng. Chem., Anal. Ed. **6**: 72.

7. NELSON, G. H., R. P. STRAKA, and MAX LEVINE.
   1939. The Effect of Temperature of Digestion, Chemical Composition, and Size of Particles on Production of Fuel Gas from Farm Wastes. J. Agric. Research, **58**: 273.

8. NORMAN, A. G., and S. H. JENKINS.
   1933. A New Method for the Determination of Cellulose Based upon Observation on the Removal of Lignin and other Incrusting Materials. Boichem. J. **26**: 818.

9. PETERSON, C. J., A. W. WALDE, and R. M. HIXON.
   1932. Effect of Temperature on Sulphuric Acid Method for Lignin. Ind. Eng. Chem., Anal. Ed. **4**: 216.

10. Standard Methods for the Examination of Water and Sewage, Seventh Edition, Lancaster Press, Lancaster, Pa.

11. SWEENEY, O. R., and L. K. ARNOLD.
   The Production of Paper from Cornstalks. Bull. 100, Iowa Eng. Expt. Sta., Iowa State College, Ames.

12. Tenney, Florence G., and S. A. Waksman.
1930. Composition of Natural Organic Materials and Their Decomposition in the Soil. V. Decomposition of Various Chemical Constituents in Plant Materials under Anaerobic Conditions. Soil Sci. **30**: 143.

NEW SPECIES OF PSYLLIDAE FROM THE WESTERN UNITED STATES

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In working over the North American Psyllidae several species have been encountered which herein are described as new. They all occur in the western part of the United States, an area in which these insects are especially abundant. I am indebted to Mr. P. W. Oman of the United States National Museum, Dr. R. H. Beamer of the University of Kansas and Mr. Joe Schuh of Oregon State College for assistance and the loan of material. The disposition of the types is indicated after each description.

Aphalara cuyama n. sp.

This extremely small species seems to resemble most the *A. artemesiae* group. Length to tip of folded wing 1.3 mm.

Color: General color greenish. Wings white with scattered brown spots.

Structure: Vertex with very prominent discal foveae, deeply cleft in front. Clypeus large, prominent. Antennae one and one third times as long as width of head.

Genitalia: Caudal lobe of male proctiger not extending beyond forceps, blunt, ventral hooks small. Forceps large, in lateral view flabellate, anterior half of apex excavate; in dorsal aspect both anterior and posterior apical margins incurved as hooks, black margined. Female genital segment quite small, shorter than remainder of abdomen.

Holotype, male, *allotype,* female, 2 male paratypes, Cuyama Ranch, California, July 25, 1935, R. H. Beamer.

Types in Snow Collection, University of Kansas, paratype in author's collection.

Aphalaroida californica n. sp.

Resembling *Aphalaroida inermis* but forewings without any glandular hairs, more rhomboidal and with a single brown macula before apex, remainder yellow or white. Length to tip of folded wings 2.00-2.25 mm.

Color: General color yellow. Forewings with irregular white areas and broad brown macula across apex.

Structure: Vertex smooth and broad. Antennae one fourth longer than width of head. Forewings very broad, flaring, somewhat rhomboidal, twice as long as greatest width.

Genitalia: Very similar to *A. pithecolobia,* male forceps broader and black margined apically.

Holotype, female, *allotype,* male, Indio, California, Aug. 5, 1935. R. H. Beamer; one female paratype Indio, California, June 29, 1933, R. H. Beamer.

Types in Snow Collection, University of Kansas.

Calophya oweni n. sp.

Resembling *Calophya californica* but vertex more rounded, genal cones very short and wings rugose and fumate. Length 2 mm.

Color: Head (except genal cones and antennae), thorax including legs (except hind tibiae), and proximal 1/7 of forewings fuscous. Remainder of body yellowish. Forewings with a narrow hyaline area proximal of the black portion.

Structure: Vertex evenly rounded downward in front. Genal cones very small, sharp. Forewings rugose and semi-opaque, evenly rounded, pterostigma very large.

Genitalia: Male proctiger almost straight-sided in lateral view, not enlarged as in other members of the genus. Forceps broad, enlarged toward apex, notched, anterior portion incurved, black tipped, apex black margined. Female genital segment slightly shorter than remainder of abdomen, valves about equal in length.

Holotype, male, *allotype,* female, 13 male and 9 female paratypes, Mesa Verde Park, Colorado, June 30, 1938, L. D. Tuthill, taken on *Phorodendron juniperinum* Engelm.

This species is named in honor of Mr. Truit L. Owen whose interest and assitance made its collection possible.

Types in author's collection, Snow Collection, and United States National Museum.

Trioza phorodendrae n. sp.

Resembling *Trioza mexicana* Crawf. but vertex rounding more evenly into genal cones, yellow in color, female genital segment shorter. Length to tip of folded wings 2.5 mm.

Color: Uniformly yellow except eyes, tip of female genital segment, ovipositor and black margins of male forceps.

Structure: Vertex somewhat rounding. Antennae about as long as width of head. Genal cones acute, somewhat divergent, two-thirds as long as vertex, pubescent. Forewings somewhat yellowish, nerves yellow. Apex of hind tibiae with three inner spines.

Genitalia: Male proctiger quite broad, anterior margin curved, posterior margin straight. Forceps in lateral aspect curving forward; "folded" so as to appear double in apical half, lateral folds evenly narrowed to apices, medial folds with heavy, black, truncate apices; bearing many very heavy medially projecting setae basally. Female genital segment as long as remainder of abdomen, rounding, produced at apex into a brown styliform portion, at least tip of ovipostitor protruding.

Holotype, male, *allotype,* female, 7 male and 17 female paratypes, Mesa Verde Park, Colorado, June 30, 1938, L. D. Tuthill.

Taken on *Phorodendron juniperinum* Engelm.

Types and paratypes in author's collection. Paratypes in Snow Collection and United States National Museum.

Trioza beameri n. sp.

Resembling *Trioza bakeri* but larger, genal cones longer and extending forward, antennae longer. Length to tip of folded wings 5 mm.

Color: The specimens at hand are uniformly whitish yellow except

the eyes and tips of the antennae. This may be due in part at least to fading.

Structure: Entire body pubescent, least prominently on adbomen. Vertex plane with two prominent sulcate depressions. Antennae about one and one-third times as long as width of head. Genal cones large, extending forward, three-fourths as long as vertex. Forewings very large, twice as long as body, acute, almost three times as long as broad, first marginal cell larger than second.

Genitalia: Male proctiger in lateral aspect broad in basal half, then obliquely truncate to apex. Forceps in lateral view almost straight to apices which are produced posteriorly into blunt black teeth; in caudal view broadest at base, bowed out, evenly narrowed to apices. Female genital segment about as long as remainder of abdomen, valves straight to acute apex, dorsal slightly longer than ventral; very pubescent.

Holotype, male, *allotype,* female, 4 male and 5 female paratypes, San Jacinto Mountains, California, July 21, 1929, R. H. Beamer.

This species is named in honor of Dr. R. H. Beamer of the University of Kansas, Department of Entomology.

Holotype, allotype and paratypes in Snow Collection, University of Kansas. Paratypes in author's collection.

Trioza forcipula n. sp.

Resembles *Trioza aylmeriae* but smaller, darker, genitalia distinct. Length to tip of folded wings, 3.0 mm.

Color: Dorsum orange-red except center of abdominal tergites, two incomplete lines on thorax, discal foveae and medial suture of vertex and antennae black. Venter black except tips of genal cones and tibiae.

Structure: Head wide, almost as wide as thorax. Discal foveae and medial suture of vertex prominent. Genal cones two thirds as long as vertex. Antennae slightly over one and one-fourth times as long as width of head. Forewings a little more than twice as long as wide, rather bluntly angled. Hind tibiae with three inner apical spines.

Genitalia: Proctiger of male with very long, upcurved posterior lobes, much as in *T. aylmeriae,* apical tuft of spines much less prominent however. Forceps bent forward and also inward, touching most of their length, apices deeply notched, anterior tooth larger. Female genital segment short, almost globose in lateral aspect, apices of valves black, slightly produced.

Holotype, male, *allotype,* female, 4 male and 29 female paratypes, Slumgullion Pass, Colorado, June 29, 1937, L. D. Tuthill; 16 male and 10 female paratypes, same data, collected by R. H. Beamer; 2 male and 2 female paratypes, Pullman, Washington, May.

Holotype, allotype, and paratypes in author's collection. Paratypes in Snow Collection, University of Kansas, and in United States National Museum.

Trioza stugma n. sp.

Resembles *T. viridis* but has short antennae genal cones are much sharper and project downward and the forewings are rounded and have a long Rs vein. Length to tip of folded wings 2.5 mm.

Color: General color green. Eyes, antennae, distal portions of leg segments, etc., light fulvous. Forewings slightly infuscated.

Structure: Head almost as wide as thorax. Vertex with two prominent foveae, very strongly protruded in front on each side of medial sulcus, overhanging the short genal cones, latter one half as long as vertex. Antennae one and one-fourth times as long as width of head. Thorax not strongly arched. Forewings rounded at apex, two and one-half times as long as wide, venation typical. Hind tibiae with two inner apical spines.

Genitalia: Male genitalia small. Proctiger short, somewhat produced caudally. Forceps very short and broad in lateral aspect, apex rounded and bearing a large, medially projecting black pointed tooth. Female genital segment short, valves subequal in length, dorsal one straight, ventral concave dorsally.

Holotype, male, *allotype,* female, Placer County, California, September, A. Koebele; 4 male paratypes, same data; one female paratype, Strawberry, California, August 8, 1929, L. D. Anderson.

Holotype, allotype and paratypes in United States National Museum. Paratypes in Snow Collection, University of Kansas, and in author's collection.

Trioza occidentalis n. sp.

This species bears a somewhat superficial resemblance to *T. tripunctata (Phyllopecta)* but may readily be distinguished from it bv the lack of wing maculation, lack of anteriorly projecting coxal spurs and by the very elongate female genitalia. Length to tip of folded wings 4 mm.

Color: The specimens at hand are golden brown, lighter ventrally.

Structure: Head narrower than thorax. Vertex quite plane, raised. Antennae not quite twice as long as width of vertex. Genal cones large, thick, conical, two-thirds as long as vertex. Thorax very strongly arched, praescutum almost projecting over pronotum. Forewings broad, blunt, two and one-half times as long as wide; marginal cells small. Hind tibiae with two inner apical spines.

Genitalia: Female genital segment two-thirds as long as remainder of abdomen, conical, upcurved.

Holotype, female, Marin County, California; one female paratype Kaslo Creek, British Columbia, A. N. Caudell. The type bears a label *Corylus rostrata* Ait. and bears the manuscript name, *"Trioza coryli."*

Holotype in United States National Museum, paratype in author's collection.

Trioza pulla n. sp.

This species resembles *T. maura* somewhat but differs from it in having much longer, more slender genal cones, longer antennae (almost twice as long as width of vertex) and in the genitalia. Length to tip of folded wings 3.5 mm.

Color: Head, thorax, femora and apical half of antennae fuscous. Remainder of legs and antennae pale. Abdomen green. Wings milky. Females lighter, vertex and thoracic dorsum fuscotestaceous.

Structure: Head broad. Vertex quite flat for Trioza. Antennae long, almost twice as long as width of vertex. Genal cones slender, acute, slightly longer than vertex, projecting downward, straight, not touching at all. Thoracic dorsum not very strongly arched. Hind tibiae with two

inner apical spines. Forewings two and one-half times as long as wide. Genal cones, legs and genitalia very pubescent.

Genitalia: Male genitalia small. Proctiger with a long blunt caudal lobe. Forceps quite broad at base, sharply narrowed midway then tapering to truncate apex with a small anterior tooth. Female genital segment about one-third as long as remainder of abdomen, dorsal valve rounding down to acute black apex, ventral valve shorter.

Holotype, male, Sumner Washington, July 6, 1935, P. W. Oman; one male paratype, same data; *allotype,* female, Vernonia, Oregon, April 21, 1936, K. Gray; 5 male and 5 female paratypes, same data.

Holotype and paratype in United State National Museum, allotype and paratypes in Oregon State College Collection, paratypes in author's collection.

Trioza inversa n. sp.

Resembles *T. frontalis* but much darker in color and male genitalia distinct. Length to tip of folded wings 3.0 mm.

Color: General color of dorsum ferrugineous with a pair of fuscous markings on vertex, a pair on scutum, another pair on scutellum. Venter, femora and antennae fuscous, remainder fulvous. Wings hyaline.

Structure: Head broad, almost as wide as thorax. Antennae one and one-third times as long as width of head. Genal cones long, quite sharp, projecting downward, four-fifths as long as vertex. Forewings only slightly angulate, almost three times as long as wide. Hind tibiae with three inner apical spines.

Genitalia: Proctiger with a basal caudal projection, apex very narrow. Forceps in lateral aspect large at base, narrowed to spatulate apices; in caudal aspect broad at base, laterally concave to apices, touching most of their length. Female genital segment about half as long as remainder of abdomen, acute, dorsal valve slightly longer than ventral.

Holotype, male, *allotype*, female, 7 male and 9 female paratypes, Slumgullion Pass, Colorado, June 29, 1937, L. D. Tuthill; 4 male and one female paratype same locality and dates, R. H. Beamer; one male and one female paratype, Logan Canyon, Utah, May 16, 1934, T. O. Thatcher; 2 male and one female paratype, Bear Lake, British Columbia, July 20-29, 1903, R. P. Currie.

Holotype, allotype and paratypes in author's collection. Paratypes in Snow Collection, University of Kansas, and in United States National Museum.

Trioza rubra n. sp.

Length to tip of folded wings 3.75 mm.

Color: The color varies through shades of red to almost black, the males being darker in general. The distal parts of the legs lighter. Antennae white except distal third black.

Structure: Vertex plane, medial sulcus prominent, bulging in front. Antennae almost twice as long as width of vertex, basal portion somewhat swollen. Genal cones large, divergent, blunt, two-thirds as long as vertex. Forewings rather bluntly angled, almost three times as long as wide. Hind tibiae with two inner apical spines.

Genitalia: Male proctiger with two caudally projecting rather sharp lobes, surrounding forceps. Forceps large at base, tapering to long slender black-tipped apices, curving forward throughout their length. Female genital segment short, about one-third as long as remainder of abdomen, dorsal valve slightly longer than ventral, dorsal valve somewhat concave, black apically.

Holotype, female, Creede, Colorado, July 9, 1937, L. D. Tuthill; *allotype,* male, Creede, Colorado, July 12, 1938, L. D. Tuthill. Paratypes as follows: 3 females same data as holotype; 4 females, Creede, Colorado, July 8, 1937, L. D. Tuthill; 2 females, Creede, Colorado, July 12, 1938, L. D. Tuthill; 2 males and 2 females, Silver City, New Mexico, July 22-23, 1936, R. H. Beamer; one male, Grand Canyon, Arizona, Aug. 11, 1927, R. H. Beamer; one male, Sun Pass, Oregon, July 1, 1935, R. H. Beamer; 2 females, Colorado, C. F. Baker.

Holotype, allotype and paratypes in author's collection. Paratypes in Snow Collection, University of Kansas, and United States National Museum.

Psylla phorodendrae n. sp.

This is a very stout species. Length to tip of folded wings 3 mm.

Color: Green, forewings olive green.

Structure: Head very broad, wider than thorax. Antennae about twice as long as width of head. Eyes borne on prominent stalk-like area of head. Genal cones short, not as long as broad, rather blunt. Thorax very broad. Wings twice as long as wide, pterostigma large.

Genitalia: Male proctiger narrow in lateral aspect, straight. Forceps very slender in lateral view, base somewhat enlarged, remainder bent forward, apices blunt, postero-apical margins sharp, black margined; in caudal aspect bowed out, apices touching, a large mesally projecting, black tipped tooth near base. Female genital segment short, about one-half as long as remainder of abdomen, dorsal valve elongate hood shaped, ventral valve very short with a large median, transparent truncate tooth.

Holotype, female, *allotype,* male, 15 female and 6 male paratypes, Huachuca Mountains, Arizona, July 18, 1938, R. H. Beamer; 2 female and 2 male paratypes, Los Angeles County, California, May, A, Koebele. The Arizona specimens were taken on mistletoe on oak, the California specimens bear the label *Phorodendron pubescens,* the latter are accompanied by several nymphs.

Holotype, allotype and paratypes in Snow Collection, University of Kansas; paratypes in United States National Museum and author's collection.

A COMPARISON OF SPRING MIGRATIONS OF SOME BIRDS THROUGH CLAY AND PALO ALTO COUNTIES, IOWA[1]

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The opportunity to observe the spring, 1938, migrating birds in Clay and Palo Alto Counties, Iowa, and reference to a list of spring, 1934, bird migration for those counties (Bennett 1938) made possible a comparison of the migrations of the two seasons. The field data were gathered in the "Ruthven area", which lies on the north-south county line of Clay and Palo Alto Counties and in the immediate vicinity of Ruthven, Iowa. That area contains a large remnant of potholes, lakes, and marshes which were free of ice March 21, 1938.

The 1938 observations were made from March 20 to June 15.

The census of the birds was made by taking a direct count of small numbers to 100 and by estimating the flocks larger than 100 individuals. Each day the species and the number of birds observed were recorded. In this way the dates of the main flight and the total number of each bird species migrating through Clay and Palo Alto Counties in 1938 was determined. Field glasses were used to spot-check flocks and rafts of birds. Each day approximately 30 miles of roads surrounding the important water areas were traversed, and observations were made from a motor boat with which the writer traveled over the larger bodies of water. Observations were made during all periods of the day, especially late in evening and early in morning when the birds appeared to be more active. Of those birds showing a distinct difference between the male and the female, sex ratios were taken.

The research was under the direction of Dr. George O. Hendrickson, Assistant Professor, Wildlife Management, and Thomas G. Scott, Associate Biologist, United States Bureau of Biological Survey.

Table 3 presents a comparison of the spring bird migration of 1938 with the spring migration of 1934 through the Ruthven area. In addition to the dates when the first and last migrants were observed and the period or date when the main flight took place, the approximate numbers of birds observed during both seasons are given in this table. Many of the birds nested in northwest Iowa and are so designated by "nesting" in the column for the "Date Last Seen". Those birds which rarely occur in northwest Iowa and probably were not present in the Ruthven area during one of the seasons, are marked "X" in the table; those birds which probably were present in both seasons but not recognized are marked "XX".

The first migrant geese of each species arrived at the Ruthven area in 1938 on approximately the same dates as they did in 1934. However, the main flight of the geese through Clay and Palo Alto Counties was one to

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two weeks earlier in 1938 than in 1934. The main flight of the Blue Goose *(Chen caerulescens)* during 1934 was on April 15, and in 1938 on March 31, although this species arrived March 19 and March 22 in 1934 and 1938, respectively. The date that geese were last seen in the Ruthven area did not vary more than three or four days for the two years, with the exception of one juvenile Blue Goose observed on May 27, 1938, three weeks later than the last Blue Goose observed in 1934. This juvenile Blue Goose was observed in company with 15 Double-crested Cormorant *(Phalacrocorax auritus auritus)*. The main flight of the White-fronted Goose *(Anser albifrons albifrons)* and Common Canada Goose *(Branta canadensis canadensis)* was two and one-half weeks and one week, respectively, earlier in 1938 than in 1934.

The total number of geese migrating through Clay and Palto Alto Counties was greater in 1938 than in 1934. However, the number of Common Canada Goose and the White-fronted Goose in 1938 increased only slightly over the numbers in 1934. The Blue Goose migrating through the two counties increased from 30,000 in 1934 to 50,000 in 1938, but the Lesser Snow Goose *(Chen hyperborea hyperborea)*, which traveled in close company with the Blue Goose, decreased in numbers in those same years. The ratio of the Blue Goose to the Lesser Snow Goose migrating together was a little more than three to one.

During the 1938 spring migration, 13 of the duck species arrived in the Ruthven area during the third week of March, and two species during the first week of April, whereas, in the 1934 season 10 species arrived during the third week of March and five species during the first week of April. The most noticeable difference in migration activities was observed in the American Pintail *(Dafila acuta tzitzihoa)*. The American Pintail arrived March 21 in the Ruthven area in great numbers during the 1938 season and were at the height of migration March 21 to April 20, as compared with the 1934 main flight which lasted two full months, March 20 to May 15. The American Pintail flight was definitely over by May 1 in the 1938 season.

The Blue-winged Teal *(Querquedula discors)* arrived two and a half weeks earlier in the Ruthven area in 1938 than in 1934. Such birds as the Lesser Loon *(Gavia immer elasson)*, Pied-billed Grebe *(Podilymbus podiceps podiceps)*, Double-crested Cormorant, American Bittern *(Botaurus lentiginosus)*, Gadwall *(Chaulelasmus streperus)*, Baldpate *(Mareca americana)*, Buffle-head *(Charitonetta albeola)*, Ruddy Duck *(Erismatura jamaicensis rubida)*, American Coot *(Fulica americana americana)*, Upland Plover *(Bartramia longicauda)*, Black Tern *(Chlidonias nigra surinamensis)*, and Yellow-headed Blackbird *(Xanthocephalus xanthocephalus)*, arrived in the lake region of Ruthven one to two weeks ahead of the first migrants of the same species in 1934.

The ducks, other than those nesting in Clay and Palo Alto Counties, were last seen at a much later date in these counties in 1938 than in 1934. The Lesser Scaup Duck *(Nyroca affinis)* was found nesting in the marshes of northwest Iowa in 1938 and remained on the lake and marshes in flocks to 30 in number until June 10, but in 1934 it was not seen after April 25. In 1938 the Ring-necked Duck *(Nyroca collaris)* remained in northwest Iowa two weeks, the Buffle-head three weeks, the Green-winged Teal *(Nettion carolinense)* and Baldpate four weeks and the Gadwall six weeks, longer than in 1934.

The majority of the ducks migrating through Ruthven increased in numbers in the 1938 season over the 1934 season. Those ducks making the most noticeable increase in migrating numbers were Redhead *(Nyroca americana)*, Ruddy Duck, Blue-winged Teal, Shoveller *(Spatula clypeata)*, Lesser Scaup Duck, and Buffle-head. Approximately 300 per cent more Redhead, 200 per cent more Ruddy Duck and Lesser Scaup Duck, 400 per cent more Buffle-head, and 66 per cent more Canvas-back *(Nyroca valisneria)* migrated through the Ruthven area in 1938 than were reported in 1934. However, about 50 per cent as many American Pintail and 66 per cent as many Common Mallard *(Anas platyrhynchos platyrhynchos)* and Gadwall migrated through the Ruthven area during 1938 as in 1934.

The number of the Double-crested Cormorant migrating through the Ruthven area in 1938 was 350 as compared with 24 individuals in 1934. Flocks of 15 to 30 Double-crested Cormorant were common sights during the last days in May and early June of 1938.

The Greater Prairie Chicken *(Tympanuchus cupido americanus)* wintering in a 1500 acre slough of Clay County numbered 4000 in the spring of 1934. A census taken in the spring of 1938 showed three individuals in the same slough.

The American Coot, according to permanent residents familiar with the bird, was reported March 14, 1938, at Greene's Slough, Clay County. The writer observed numerous American Coot on March 21. In 1934 the bird arrived at Ruthven on March 20. The American Coot remained in Clay and Palo Alto Counties after the migration season in larger numbers than any other migratory waterfowl. The number of American Coot in 1938 was 66 per cent greater than the number migrating through the Ruthven area in 1934. The Florida Gallinule *(Gallinula chloropus cachinnans)*, which in numbers were about two per cent of the American Coot, arrived April 28, 1938.

During the 1938 migration season the White Pelican *(Pelecanus erythrorhynchos)*, which had been recorded for the Sioux City area but not for the Ruthven area (DuMont, 1933) arrived at the Ruthven area on April 21 and left about June 10. They numbered approximately 500. The White Pelican spent a great deal of time feeding on the sloughs and lakes in the Ruthven area. They were also observed at Lake Okoboji in Dickinson County in the spring of 1938.

The American Egret *(Casmerodius albus egretta)* reported in Palo Alto County, 1932, (DuMont, 1933) was observed in Clay County August 8, 1938.

The shore birds did not appear to be numerous during the migration season of 1938 in the Ruthven area. Many of the shore birds reported in 1934 were not seen in 1938, including such birds as the American Golden Plover *(Pluvialis dominica dominica)*, the Black-bellied Plover *(Squatarola squatarola)*, the Marbled Godwit *(Limosa fedoa)*, the Hudsonian Godwit *(Limosa haemastica)*, and the Avocet *(Recurvirostra americana)*. The water levels were much higher in the Ruthven area for the spring months of 1938 than they had been for many years previous. These increased water levels eliminated much of the open shore lines which shore birds frequent in their spring migration.

Of those ducks showing distinct sex markings, sex ratios were taken. Flocks were counted for sex only when the observer could approach

close enough for accurate identification. With the exception of one species, the male ducks in the flocks were in excess of the female ducks during the 1938 spring migration through the Ruthven area. McIlhenny (1934) gave sex ratios of the ducks that he banded during 22 consecutive years on Avery Island, Louisiana. He found an excess of males in each species banded on this wintering ground of the ducks. Furniss (1935) reported the males in excess of the females on the duck breeding grounds of Saskatchewan, Canada. Table 1 presents a brief summary of the sex ratios of the ducks on the wintering grounds, on the spring migration through northwest Iowa, and on the breeding grounds of Saskatchewan.

TABLE 1. *Sex ratios of ducks on their wintering ground (Louisiana), spring Migration (Iowa), and breeding ground (Saskatchewan)*

	Ratios at Avery Island, Louisiana, 1934	Ratios at Ruthven, Iowa, 1938	Ratios at Saskatchewan, Canada, 1935
Species	Male to Female	Male to Female	Male to Female
Common Mallard *(Anas platyrhynchos platyrhynchos)* ..	—— ——	1.0 — 1	1.7 — 1
Gadwall *(Chaulelasmus streperus)*	—— ——	1.5 — 1	1.5 — 1
Baldpate *(Mareca americana)*	—— ——	2.0 — 1	1.4 — 1
American Pintail *(Dafila acuta tzitzihoa)*	1.66 — 1	3.0 — 1	2.9 — 1
Green-winged Teal *(Nettion carolinense)*	—— ——	2.0 — 1	1.2 — 1
Blue-winged Teal *(Querquedula discors)*	4.2 — 1	1.5 — 1	1.5 — 1
Shoveller *(Spatula clypeata)*	—— ——	2.0 — 1	1.0 — 1
Redhead *(Nyroca americana)*	—— ——	1.5 — 1	1.2 — 1
Ring-necked Duck *(Nyroca collaris)*	4.5 — 1	1.5 — 1	1.0 — 1
Canvas-back *(Nyroca valisneria)*	4.5 — 1	2.0 — 1	1.5 — 1
Lesser Scaup Duck *(Nyroca affinis)*	2.5 — 1	2.0 — 1	1.6 — 1
Buffle-head *(Charitonetta albeola)*	—— ——	1.4 — 1	2.0 — 1
Ruddy Duck *(Erismatura jamaicensis rubida)*	—— ——	1.25 — 1	3.4 — 1
American Golden-eye *(Glaucionetta clangula americana)* ...	—— ——	—— ——	1.1 — 1
Averages	3.47 — 1	1.74 — 1	1.64 — 1

The state of weather at any particular point along the migration route has little, if anything, to do with the time of arrival of migratory birds, but the weather at various points on the route may affect the activities of the birds as they pass through these particular spots on their journey north by influencing the food supply (Lincoln, 1935). Table 2

TABLE 2. *Average and departure from the average temperature and rainfull for the State of Iowa, Clay and Palo Alto Counties for 1934 and 1938*

(Data for this table taken from Climatological Data of the Iowa Section of the United States Department of Agriculture Weather Bureau.)

Month	1934				1938				Difference	
	Average Temperature	Departure from Average	Average Precipitation	Departure from Average	Average Temperature	Departure from Average	Average Precipitation	Departure from Average	The Positive Sign (+) indicates Values larger in 1938 than in 1934	
									Temp.	Prec.
Iowa:										
March	34.4	+ 0.1	1.09	– 0.65	43.7	+ 9.2	2.35	+ 0.62	+ 9.3	+ 1.26
April	50.4	+ 1.7	1.07	– 1.67	50.3	+ 1.6	3.66	+ 0.93	– 0.1	+ 2.59
May	69.6	+ 9.6	1.02	– 3.06	59.5	– 0.5	5.45	+ 1.36	– 10.1	+ 4.43
June	77.2	+ 7.6	3.49	– 1.12	69.2	– 0.3	4.67	+ 0.06	– 8.0	+ 1.18
Clay County:										
March	32.2	+ 1.3	0.86	– 0.35	39.6	+ 8.7	1.51	+ 0.34	– 7.4	+ 0.65
April	48.8	+ 2.2	1.97	– 1.20	49.0	+ 2.4	2.89	+ 0.27	+ 0.2	+ 0.92
May	70.6	+ 11.5	0.24	– 3.40	57	– 2.1	6.08	+ 1.72	– 13.4	+ 5.84
June	74.0	+ 5.9	7.80	+ 4.28	69.2	+ 1.1	5.39	+ 1.87	– 4.8	– 2.41
Palo Alto County:										
March	32.2	+ 1.0	1.42	+ 0.08	39.8	+ 8.6	1.67	+ 0.48	+ 7.6	+ 0.25
April	47.8	+ 1.2	1.20	– 1.51	47.6	+ 1.0	2.60	+ 0.16	– 0.2	+ 1.40
May	69.6	+ 10.5	0.30	– 3.53	55.4	– 3.7	4.27	+ 0.21	– 14.2	+ 3.97
June	73.8	+ 5.7	5.00	+ 0.85	67.6	– 0.5	4.78	+ 0.61	– 6.2	– 0.22

TABLE 3. *Comparison of the bird migration seasons of 1934 and 1938 in Clay and Palo Alto Counties, Iowa*

Species	Date First Seen		Main Flight		Date Last Seen		Number Seen	
	1934	1938	1934	1938	1934	1938	1934	1938
Lesser Loon *(Gavia immer elasson)*	Apr. 10	Apr. 2	....	Apr. 1-3	....	Apr. 5	30	25
Eared Grebe *(Colymbus nigricollis californicus)*	Apr. 20	Apr. 25	....	May 14	Nesting*	Nesting	85	150
Pied-billed Grebe *(Podilymbus podiceps podiceps)*	Apr. 14	Mar. 22	Apr. 29	Apr. 15	Nesting	Nesting	800	1,500
White Pelican *(Pelecanus erythrorhynchos)* ..	...†...	Apr. 21	....	Apr. 28	....	June 10	....	500
Double-crested Cormorant *(Phalacrocorax auritus auritis)*	Apr. 30	Apr. 11	....	May 2	....	May 30	24	350
American Egret *(Casmerodius albus egretta)* ..	...†...	Aug. 12	....	1 ind. seen	....	Aug. 13	....	1
Black-crowned Night Heron *(Nycticorax nycticorax hoactli)*	...†...	Apr. 13	....	Apr. 25	....	Nesting	....	300
American Bittern *(Botaurus lentiginosus)*	Apr. 22	Apr. 13	May 15-19	Apr. 23	Nesting	Nesting	600	500
Eastern Least Bittern *(Ixobrychus exilis exilis)*	...‡...	Apr. 26	....	May 24	....	Nesting	....	2,000
Common Canada Goose *(Branta canadensis canadensis)*	Mar. 19	Mar. 17	Apr. 17	Apr. 10	May 5	Apr. 26	5,000	7,000
White-fronted Goose *(Anser albifrons albifrons)*	Apr. 6	Mar. 26	Apr. 12	Mar. 26-Apr. 4	Apr. 30	Apr. 29	200	300
Lesser Snow Goose *(Chen hyperborea hyperborea)*	Mar. 19	Mar. 22	Apr. 16	Apr. 10	May 2	Apr. 30	20,000	15,000
Blue Goose *(Chen caerulescens)*	Mar. 19	Mar. 22	Apr. 15	Mar. 31	May 1	May 27	30,000	50,000

* Nesting birds—last migrants not detected.
† Rare birds, probably not present in this year.
‡ Birds probably present in this year but not recorded.

TABLE 3. (Continued)

Species	Date First Seen		Main Flight		Date Last Seen		Number Seen	
	1934	1938	1934	1938	1934	1938	1934	1938
Common Mallard *(Anas platyrhynchos platyrhynchos)* .	Mar. 15	Mar. 21	Mar. 20-May 15	Mar. 20-Apr. 15	Nesting	Nesting	400,000	250,000
Red-legged Black Duck *(Anas rubripes rubripes)*	Mar. 20	...‡...	....	....	....	....	100	......
Gadwall *(Chaulelasmus streperus)*	Apr. 4	Mar. 24	Apr. 19	Mar. 24	Apr. 28	Aug. 12	10,000	8,000
Baldpate *(Mareca americana)*	Apr. 2	Mar. 24	Apr. 19	Apr. 8	Apr. 28	June 4	8,000	10,000
American Pintail *(Dafila acuta tzitzihoa)*	Mar. 15	Mar. 21	Mar. 20-May 15	Mar. 21-Apr. 20	Nesting	Nesting	1,000,000+	500,000
Green-winged Teal *(Nettion carolinense)*	Mar. 20	Mar. 26	Apr. 14	Apr. 18	Apr. 28	May 28	8,000	10,000
Blue-winged Teal *(Querquedula discors)*	Apr. 7	Mar. 21	Apr. 25	Apr. 19	Nesting	Nesting	20,000	30,000
Shoveller *(Spatula clypeata)*	Mar. 23	Mar. 21	Apr. 13	Apr. 15	Nesting	Nesting	15,000	25,000
Redhead *(Nyroca americana)*	Mar. 20	Mar. 22	Apr. 8	Mar. 24-Apr. 18	Nesting	Nesting	1,000	3,500
Ring-necked Duck *(Nyroca collaris)*	Mar. 20	Mar. 23	Apr. 12	Mar. 28-Apr. 1	May 1	May 12	8,000	10,000
Canvas-back *(Nyroca valisneria)*	Mar. 15	Mar. 22	Apr. 9	Apr. 18	Nesting	Nesting	300	500
Lesser Scaup Duck *(Nyroca affinis)*	Mar. 15	Mar. 21	Apr. 13-17	Apr. 6-12	Apr. 25	Nesting	20,000	40,000
American Golden-eye *(Glaucionetta clangula americana)* ..	Mar. 19	Apr. 3	Mar. 31	Apr. 3	Apr. 13	Apr. 13	300	30
Buffle-head *(Charitonetta albeola)*	Apr. 9	Mar. 23	Apr. 13	Apr. 1	Apr. 13	May 9	50	200
Ruddy Duck *(Erismatura jamaicensis rubida)*	Apr. 14	Apr. 2	Apr. 18	Apr. 29	Nesting	Nesting	500	1,000
Hooded Merganser *(Lophodytes cucullatus)*	Apr. 10	Apr. 3	....	May 10	....	....	40	35

TABLE 3. (Continued)

Species	Date First Seen		Main Flight		Date Last Seen		Number Seen	
	1934	1938	1934	1938	1934	1938	1934	1938
American Merganser (*Mergus merganser americanus*)	Mar. 18	...‡...	Apr. 10	....	Apr. 20	....	100	....
Red-breasted Merganser (*Mergus serrator*)	Mar. 18	Apr. 11	....	Apr. 11	....	Apr. 20	60	70
Sharp-shinned Hawk (*Accipiter velox velox*)	...‡...	Apr. 15	....	....	....	....	....	....
Cooper's Hawk (*Accipiter cooperi*)	...‡...	Apr. 15	....	....	....	....	....	....
Eastern Red-tailed Hawk (*Buteo borealis borealis*)	Mar. 23	Mar. 28	....	....	Nesting	Nesting	20	10
Marsh Hawk (*Circus hudsonius*)	Mar. 22	Mar. 30	Apr. 10	Apr. 5	Nesting	Nesting	200	100
Osprey (*Pandion haliaëtus carolinensis*)	Apr. 30	...†...	....	....	May 2	....	2	....
Duck Hawk (*Falco peregrinus anatum*)	Apr. 13	...†...	....	....	May 3	....	3	....
Western Pigeon Hawk (*Falco columbarius bendirei*) ..	Mar. 23	...†...	only 1 seen	....	....	....	1	....
Earstern Sparrow Hawk (*Falco sparverius sparverius*) .	Apr. 6	Apr. 1	....	....	Nesting	Nesting	500	....
Greater Prairie Chicken (*Tympanuchus cupido americanus*)	Winter-ing §	Apr. 15 Winter-ing	Mar. 20	....	Apr. 6	Apr. 15	4,000	3 ind. seen once

§ Birds present all winter—first arrivals not evident.

TABLE 3. (Continued)

Species	Date First Seen		Main Flight		Date Last Seen		Number Seen	
	1934	1938	1934	1938	1934	1938	1934	1938
Virginia Rail (*Rallus limicola limicola*)	...‡...	May 6	....	May 6	....	Nesting	....	1,500
Sora (*Porzana carolina*)	...‡...	Apr. 26	....	May 6	....	Nesting	....	1,000
Forida Gallinule (*Gallinula chloropus cachinnans*)	...‡...	Apr. 26	....	May 16	....	Nesting	....	1,500
American Coot (*Fulica americana americana*)	Mar. 20	Mar. 14	Apr 23-25	Apr. 24	Nesting	Nesting	30,000	50,000
Piping Plover (*Charadrius melodus*)	May 3	...‡...	May 3-19	....	May 19	....	11	....
Semipalmated Plover (*Charadrius semipalmatus*)	May 2	...‡...	May 14	....	June 5	....	200	....
Killdeer (*Oxyechus vociferus vociferus*)	Mar. 16	Mar. 23	April	Mar. 25-Apr. 15	Nesting	Nesting	500	500
American Golden Plover (*Pluvialis dominica dominica*) .	May 7	...‡...	May 7	....	May 28	....	14	....
Black-bellied Plover (*Squatarola squatarola*)	Apr. 27	...‡...	May 28	....	June 1	....	41	....
Ruddy Turnstone (*Arenaria interpres morinella*)	May 11	...‡...	May 22	....	June 2	....	52	....
Wilson's Snipe (*Capella delicata*)	Apr. 22	Mar. 26	Apr. 24-May 2	Apr. 22-May 2	May 16	May 10	500	400
Upland Plover (*Bartramia longicauda*)	May 5	Apr. 22	May 11	May 5	Nesting	Nesting	35	75
Spotted Sandpiper (*Actitis macularia*)	May 1	Apr. 26	May 22	May 12	Nesting	Nesting	75	200
Eastern Solitary Sandpiper (*Tringa solitaria solitaria*)	May 21	...‡...	May 22	....	June 1	....	40	....

TABLE 3. (Continued)

Species	Date First Seen		Main Flight		Date Last Seen		Number Seen	
	1934	1938	1934	1938	1934	1938	1934	1938
Western Willet (*Catoptrophorus semipalmatus inornatus*)	May 1	May 17	May 7-11	1 indiv. seen	May 16	May 17	6	1
Greater Yellow-legs (*Totanus melanoleucus*)	Apr. 6	Apr. 10	Apr. 18-28	Apr. 26	May 5	May 10	150	175
Lesser Yellow-legs (*Totanus flavipes*)	Apr. 8	Apr. 22	Apr. 28	Apr. 26	June 3	May 17	300	500
American Knot (*Calidris canutus rufus*)	May 21	...‡...	1 flock of 14 seen	....	....	....	14	....
Pectoral Sandpiper (*Pisobia melanotos*)	Apr. 2	...‡...	May 21	....	June 2	....	800	....
White-rumped Sandpiper (*Pisobia fuscicollis*)	May 12	...‡...	May 22	....	June 3	....	400	....
Baird's Sandpiper (*Pisobia bairdi*)	May 10	...‡...	May 14	....	May 23	....	250	....
Least Sandpiper (*Pisobia minutilla*)	Apr. 19	...‡...	May 21	....	June 1	....	500	....
Red-backed Sandpiper (*Pelidna alpina sakhalina*)	May 7	...‡...	May 22-24	....	June 7	....	1,000	....
Dowitcher (*Limnodromus griseus*)	May 10	...‡...	May 14	....	May 22	....	133	....
Stilt Sandpiper (*Micropalama himantopus*)	May 7	...‡...	May 24	....	June 7	....	300	....
Semipalmated Sandpiper (*Ereunetes pusillus*)	May 3	...‡...	May 15	....	June 3	....	1,000	....
Western Sandpiper (*Ereunetes maurii*)	May 22	...‡...	3 were seen on this date	....	....	....	3	....

TABLE 3 (Continued)

Species	Date First Seen		Main Flight		Date Last Seen		Number Seen	
	1934	1938	1934	1938	1934	1938	1934	1938
Marbled Godwit (*Limosa fedoa*)	Apr. 28	...‡...	May 14	....	June 2	....	41	....
Hudsonian Godwit (*Limosa haemastica*)	Apr. 13	...‡...	May 13-14	....	May 26	....	81	....
Sanderling (*Crocethis alba*)	May 5	...‡...	May 23	....	June 5	....	28	....
Avocet (*Recurvirostra americana*)	May 13	...‡...	....	....	June 28	....	2	....
Wilson's Phalarope (*Steganopus tricolor*)	Apr. 22	May 6	May 15	May 10	May 24	May 25	144	150
Northern Phalarope (*Lobipes lobatus*)	May 11	...‡...	May 21	....	May 29	....	250	....
Parasitic Jaeger (*Stercorarius parasiticus*)	May 28	...†...	....	....	....	....	1	....
Herring Gull (*Larus argentatus smithsonianus*)	Mar. 15	Mar. 21	Apr. 14	Apr. 15	May 10	May 9	1,000	1,000
Ring-billed Gull (*Larus delawarensis*)	Mar. 16	Mar. 21	Apr. 17	Apr. 15	May 8	May 9	600	500
Franklin's Gull (*Larus pipixcan*)	Apr. 11	Apr. 15	Apr. 21-25	May 9	May 12	June 4	60,000	150,000
Bonaparte's Gull (*Larus philadelphia*)	Apr. 13	Apr. 15	Apr. 22	Apr. 28	May 10	May 26	40,000	75,000
Forster's Tern (*Sterna forsteri*)	Apr. 18	Apr. 15	May 1-10	May 1	Nesting	Nesting	1,500	2,000
Black Tern (*Chlidonias nigra surinamensis*)	May 12	Apr. 20	May 15-20	May 10	Nesting	Nesting	3,000	3,500

TABLE 3 (Continued)

Species	Date First Seen		Main Flight		Date Last Seen		Number Seen	
	1934	1938	1934	1938	1934	1938	1934	1938
Short-eared Owl (*Asio flammeus flammeus*)	Mar. 19	Mar. 21	....	....	Nesting	Nesting	20	....
Eastern Belted Kingfisher (*Megaceryle alcyon alcyon*) ...	Apr. 5	Apr. 18	....	....	Nesting	....	300	250
Bank Swallow (*Riparia riparia riparia*)	...‡...	Apr. 28	....	Apr. 30	....	Nesting	....	1,200
Northern Cliff Swallow (*Petrochelidon albifrons albifrons*)	...‡...	Apr. 21	....	Apr. 21	....	Apr. 28	....	500
Purple Martin (*Progne subis subis*)	...‡...	Mar. 24	....	Apr. 15	....	Nesting	....	....
Northern Bue Jay (*Cyanocitta cristata cristata*) ..	...‡...	Apr. 30	....	Apr. 30	....	Nesting	....	250
Short-billed Marsh Wren (*Cistothorus stellaris*)	‡...	May 2	....	May 15	....	Nesting	....	3,500
Eastern Bluebird (*Sialia sialis sialis*)	Mar. 17	Mar. 30	....	Apr. 15	Nesting	Nesting	40	35
Yellow-headed Blackbird	Apr. 8	Mar. 22	Apr. 10-20	Apr. 15	Nesting	Nesting	100,000	150,000
Red-wing (*Agelaius phoeniceus*)	Mar. 19	Mar. 21	Apr. 13	Apr 7	Nesting	Nesting	500,000	500,000
Rusty Blackbird (*Euphagus carolinus*)	Mar. 21	Mar. 21	....	Apr. 15	....	....	5,000	50,000
Brewer's Blackbird (*Euphagus cyanocephalus*)	...‡...	Mar .21	....	Apr. 7	....	....	10,000	10,000
Bronzed Grackle (*Quiscalus quiscula aeneus*) ...	Mar. 21	Mar. 21	....	Apr. 7	Nesting	Nesting	500,000	300,000

Note: Birds for which the data are incomplete either were not observed or have not been identified.

gives a comparison of the temperature and the precipitation with their variations from the average for the state of Iowa, and Clay and Palo Alto Counties, for the period March—June, in 1934, with that in 1938. The last two columns give the temperature difference in degrees Fahrenheit and the precipitation difference in inches of rainfall between the two years by monthly periods.

The average temperature for the state and Clay County for March, 1938, was 9.3 and 7.4 degrees (F.), respectively, above the temperature for the same month in 1934 (table 2). May, 1938, was 10.1 and 13.4 degrees cooler for the state and Clay County, respectively, than for the same month in 1934. The precipitation for the months March—May, 1938, was higher than for the corresponding months in 1934, for Clay and Palo Alto Counties and the entire state. The state as a whole received 9.46 inches and Clay County 5 inches more rain for the months March—June, 1938, than for the corresponding months in 1934.

In general, the four months, March—June, 1938, were unusually wet and cool with above normal rainfall for the entire period, while the 1934 migration season had temperature high above normal and rainfall below normal during the entire period.

A factor which might have had a bearing upon the lengths of time that the migrants remained in the area in 1938, but not upon the date of first arrival or the height of the migration, was the greater abundance of water in many of the potholes and other shallow feeding areas of the waterfowl. This increase of water probably acted as an incentive to the ducks to remain longer in the Ruthven area by offering more food and protection. Another factor, probably causing the birds to remain longer in the area in 1938, was the abnormally cool weather for the months of May and June. The temperature for these same two months of 1934 was far above average, and was combined with a general drought.

SUMMARY

1. Spring bird migration into Clay and Palo Alto Counties, Iowa, started approximately March 20, 1934 and 1938.

2. Five of the duck species arrived in the Ruthven area one to two weeks earlier in 1938 than in 1934, and the four geese species arrived two to 10 days earlier in 1938 than in 1934.

3. The total number of migrating geese and the number of migrating Redhead, Ruddy Duck, Blue-winged Teal, Shoveller, Lesser Scaup Duck, and Buffle-head in 1938 was greater than in 1934, whereas, the number of migrating Common Mallard, Gadwall, and American Pintail in 1938 decreased from their 1934 numbers.

4. The American Egret and the White Pelican, both rare birds in northwest Iowa, were observed in the Ruthven area in 1938.

5. Shore birds were fewer in 1938 than in 1934 for the water levels were higher and consequently there were fewer open shore lines for the shore birds.

6. Clay and Palo Alto Counties, March—June, 1938, received more rainfall than in the corresponding months, 1934.

7. The temperature at the Ruthven area was above average at the time of arrival of the first migrants in that area, but cooled off to below

normal temperature in May and June, 1938, whereas, temperatures far above average were recorded throughout the whole length of the migration season in 1934.

LITERATURE CITED

BENNETT, LOGAN J.
1938. The 1934 Spring Migration of Some Birds Through Clay and Palo Alto Counties, Iowa. Iowa Bird Life, **8**: 2-6.

DUMONT, PHILIP A.
1933. A Revised List of the Birds of Iowa. University of Iowa Studies in Natural History, **15**: 1-171.

FURNISS, O. C.
1935. The Sex Ratio in Ducks. The Wilson Bulletin, **47**: 277-278.

LINCOLN, FREDRICK C.
1935. The Migration of North American Birds. U. S. Dept. Agric. Circ. No. 363, 72.

MCILHENNY, E. A.
1934. Twenty-two Years of Banding Migratory Wild Fowl at Avery Island, Louisiana. The Auk, **51**: 328-337.

REED, CHARLES D.
1934. Monthly Report, U. S. Dept. of Agric. Weather Bureau, Iowa Section, **45**: 17-48.

———.
1938. Monthly Report. U. S. Dept. of Agric. Weather Bureau, Iowa Section, **49**: 17-50.

THE CICINDELIDAE OF IOWA (COLEOPTERA)

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The Cicindelidae, or "tiger beetles," of Iowa present an attractive and interesting group of Coleoptera to the amateur collector or the student of ecology, but a challenge to the taxonomist. The collector finds sport in the capture of specimens and is pleased by the ease and certainty with which they may be preserved in all their beautiful colors. Students of ecology have discovered that the plastic nature of color and markings of certain Cicindela furnish peculiarly appropriate material for the study of variations induced by climatic and physical factors. The taxonomist confronted with the problem of naming members of this variable group must discard pervious conceptions of the "fixity of species" and broaden the classification to include every step of gradation in the transition of one form to another.

The fully established fact that color variation in this genus is so extensive that one can scarcely discern at which point parent stocks begin and end has made definite conclusions regarding some few species impractical or almost impossible. The genitalia characters frequently used to determine species among other groups are of little value when applied to Cicindela. Considerable time was spent studying the habits of these insects; and the author's conclusions are based upon observations made and data collected.

The present study is limited to the genera Tetracha and Cicindela, in the former genus there has been only one species collected, the latter contributing a total of twenty species and nine varieties, including six species whose probable occurrence in Iowa is indicated by distribution records.

The number of species of the two genera found in Iowa compares favorably with published records of Minnesota tiger beetles by Dawson (1928) and the Indiana species reported by Montgomery (1930). It is difficult in some instances to refrain from including a few additional species whose records indicate that the distribution almost of necessity must place them within the state.

A majority of the species are confined to peculiar geological or soil formations and consequently their occurrence is limited. The forms that are confined to sandy or clay soils must be collected in such special environments as provide these soil types. Trips have been made to various parts of the state throughout the past two years with special reference to these peculiar types of soil in which the forms must be expected to exist.

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All members of the family are entirely predacious, quite aggressive in character toward small or defenseless insects, thereby earning the name tiger beetle. This inference is highly complimentary because adults readily lose their tiger-like attitude if the intended victim exhibits courageous resistance. The situations inhabited by both adults and larvae render their predations of little economic value but the interest long centered upon this group encourages continued study.

It is hoped that this paper will aid the student to classify species of Cicindelidae collected in Iowa and promote interest in obtaining further distribution records.

HISTORICAL REVIEW

The study of the genus Cicindela and its representatives in Iowa has been rendered exceedingly difficult because of the inadequate keys and many inaccurate determinations found in the collections which the author has had an opportunity to examine. The species have been found most difficult to separate, and constant study has resulted in innumerable shiftings of material and names. One can immediately infer such a condition from the multitude of species and varieties which have been described by systematists, and relegated to synonymy or insignificance by workers in the last two decades. Horn (1930) verified the inevitable synonymy of a large percentage of Casey's work which had been suspected for some time.

In the list of Iowa Coleoptera by Wickham (1911) the Cicindela number fourteen species and seven varieties. Casey (1909) described *C. levettei* from Iowa material, and (1913) *cuprascens* subsp. *mercurialis* from one male specimen. Leng (1920) places *levettei* as a variety of *sexguttata* Fabr. The author considers *levettei* as a synonym, and for purposes of keying, raises *tridens* Csy. to varietal rank. In the case of *cuprascens* subsp. *mercurialis* the author agrees with Horn (1930) in placing this as a synonym of *cuprascens* var. *macra* Lec. Thus the published records of Iowa species other than those published by Wickham have been relegated to oblivion. King (1914) published some Cicindelidae records for Henry County, but Professor Jaques has not felt free to verify these in view of the absence of corroborating evidence.

Family Cicindelidae

Key to Genera

1. Third joint of maxillary palpi elongate, longer than the fourth or terminal joint .... **Tetracha**

   Third joint of maxillary palpi shorter than, or equal to the fourth joint .... **Cicindela**

Genus TETRACHA Hope

1838 Hope, The Coleopterist's Manual, vol. 2, p 6.

Large metallic green species; third joint of maxillary palpi elongate, longer than the fourth or terminal joint; appendages usually lighter colored than ventral body surface; prothorax broader than long, wider anteriorly than the head, glabrous; head large, glabrous, except for a few orbital bristles; eyes circular; labrum short. Elytra deeply punctate, subparallel, slightly convex.

Three basal joints of anterior tarsi of male dilated, pubescent beneath. Anterior tarsi of female simple.

Members of this group are nocturnal, hiding by day, hunting at night.

KEY TO SPECIES

1. Color dark green or brownish green, elytra without markings; appendages dark brown .... *virginica*
   Color light golden or brassy green, elytra with large, luteous apical lunules; appendages testaceous .... *carolina*

TETRACHA VIRGINICA Linn.

1776 *Cicindela virginica* Linnaeus, Systema Naturae, ed. 12, vol. 1, p 657.
1778 *Cicindela virginata* Linnaeus, Systema Naturae, ed. 13, vol. 1, p 1922.
1920 *Tetracha virginica* Leng, Cat. Coleop. Amer., p 40.
1930 *Megacephala (Tetracha) virginea* Horn, Trans. Amer. Ent. Soc., vol. 56, p 79.

Length 17 to 22 mm. Head and thorax dark metallic green; elytra dark brownish black with a broad, green, marginal band; antennae, mouth parts, legs and caudal abdominal segment ferrugineous. Elytra coarsely and deeply punctate, supporting a few feeble setae; body glabrous below, only a few short bristles present on the appendages. Prothorax prominent, broader than long, widest at anterior end. Head smooth, narrower than the anterior part of the thorax; eyes small and round.

This large, somewhat clumsy appearing beetle is found in southern Iowa. Entirely nocturnal in habit, it is seldom collected except at artifical lights. During the day it hides under stones and logs in rather open damp places usually along the banks of water courses, often frequenting extremely muddy places, sometimes hiding in the cracks of sunbaked clay. It has been observed on the sidewalks in the cities of Ottumwa and Burlington leisurely hunting prey in the deeper shadows close to bright patches of illumination.

Apparently the life history is not known. The adults are found throughout July, August and occasionally in early September.

County records: Appanoose, VIII, 1930 (Ware), VIII, IX, 1930 (Russell); Davis, VII, 1933 (Travis); Decatur, VII, 1931 (Loudon); Des Moines, VIII, 1926 (H. M. Harris), VIII, 1932 (Moore), VII, (Dodds); Henry, VI, 1934 (Millspaugh), July and August, frequent; Jefferson, VII, 22, 1935; Lee, IX, 1916 (Jaques); Monroe, VIII, 1932 (Barker), VII, 1933 (Dodds); Scott, LeClaire, (Rolfs); Story, Ames (very old labels without year date); Van Buren, VIII, IX, 1930 (Rambo); Wapello, VII, VIII, 1932 (Moore), VII, 1936 (Eckhoff).

Genus CICINDELA Linn.

1758 Linnaeus, Systema Naturae, ed. 10, vol. 1, p 407.

Third joint of maxillary palpi shorter than, or equal to, the fourth or terminal joint. Eyes prominent, oval; form usually slender; elytra convex and subparallel at the sides; legs long and slender. Minor structural differences such as punctation, pubescence, formation of elytral outline, labium, thorax, variance of color and markings present characters useful in separating the species.

The male sex can be distinguished by the three basal joints of

anterior tarsi dilated and densely pilose beneath, and the sixth ventral segment broadly emarginate.

Female tarsi are slender; last ventral segment longer, slightly compressed at sides with a longitudinal impression on the posterior half and a stronger elytral emargination in species exhibiting that character.

A majority of the species live in sunny places, on sandy roads and fiends, clay ravines, lake shores, river banks and mud flats. The adults are very active; they run or hide with great facility and take wing upon slight provocation.

The larvae occur in the same local habitat as the adult beetles. For the most part they dig essentially perpendicular burrows in which they live and secure their food by waiting at the mouth of the burrow until some unsuspecting insect pedestrian happens by, whereupon it is suddenly seized, dragged into the burrow and devoured. The larva is protected by the armor on top of the flattened head and thorax. A pair of strong hooks situated on the dorsal surface of the fifth abdominal segment enables it to maintain a strategic position at the mouth of the burrow without much danger of being ignobly dislodged by some large victim.

KEY TO IOWA CICINDELA

The key includes species that have been available for examination of both Iowa specimens and those collected near the borders of the state. Three species with quite variable characters have been keyed out in more than one place. The term marking is applied to the unpigmented white pattern present on the elytra. Complete markings indicate a pattern with an entire humeral lunule, marginal band, middle band and entire apical lunule (figured in Plate I).

1. Humeral angles of elytra distinct, angulate 3

   Elytra narrowed at base, humeral angles rounded 2

2. Elytra thinly covered with pale, decumbent hairs; legs greenish or bronzed .... *celeripes*

   Elytra bare; legs testaceous .... *cursitans*

3. Abdomen not red 4

   Abdomen red .... *rufiventris*

4. Thoracic pubescence erect or lacking 5

   Thoracic pubescence decumbent 29

5. Labrum normal, width at least 2 times the length 7

   Labrum elongate, width less than 2 times the length 6

6. Body sparsely pubescent beneath; front with a distinct depression or excavation between the eyes .... *longilabris*

   Body glabrous beneath; front normal .... *unipunctata*

7. Labrum distinctly tridentate (rarely with only 2 teeth) 8

   Labrum unidentate or not dentate at all 25


formosa
generosa
lengi
longilabris
purpurea
limbalis
splendida
-guttata
repanda
hirticollis
sexguttata
patruela
tranquebarica
unipunctata
cuprascens
macra
lecontei
lecontei
fulgida
punctulata
punctulata
lepida
rufiventris
celeripes
humeral lunule
marginal band
middle band
apical lunule
complete markings
basal dot
humeral dot
posthumeral dot
extra
marginal dot
discal dot
anteapical dot
apical dot
incomplete markings

8. Front bare; orbital bristles scarce, weak 9
   Front pubescent or with strong prominent orbital bristles* 13

9. Color green, bluish, or violaceous 10
   Color opaque mossy brown ....*unipunctata*

10. Color green, often with bluish or purplish tints 11
    Color deep purple, no greenish tint ....*sexguttata* var. *violaceae*

11. Body scantily haired or glabrous beneath; elytra without middle band 12
    Body hairy beneath; elytra with middle band or more than 8 dots ....*patruela*

12. Elytra with 6 or 8 distinct spots ....*sexguttata*
    Elytra immaculate or with not more than 4 spots ....*sexguttata* var. *tridens*

13. Markings complete, all connected by the marginal line 14
    Markings not entirely connected by the marginal line ....16 (rarely confluent in *scutellaris* var. *lecontei* which lacks the middle band)

14. Length more than 14.5 mm., form robust 15
    Length less than 14.5 mm.; form slender, elytra granulate, not punctate; humeral lunule obliquely elongated ....*lengi*

15. Elytra impunctate or with few punctures; thorax cupreous, usually bare dorsally. Middle band nearly transverse ....*formosa*
    Elytra densely punctate with shallow green or blue punctures, middle band deflexed, often hooked at tip ....*formosa* var. *generosa*

16. Humeral lunule complete, obliquely elongated, terminating closer to the suture than the margin 17
    Humeral lunule incomplete (shortened), represented by small spots or entirely absent 18

17. Color above brilliant to blackish red; elytra strongly and coarsely punctured ....*fulgida*
    Color above greenish brown to very dark brown; elytra moderately punctate with blue punctures ....*tranquebarica*

18. Elytra granulate punctate or granulate 19
    Elytra impunctate or very feebly punctate; color above cupreous red to greenish bronze ....*scutellaris* var. *lecontei*

19. Head, thorax and elytra of the same color dorsally 20
    Head and thorax blue or green; elytra red or cupreous 24

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* Female *scutellaris* var. *lecontei* may lack frontal pubescence except for orbital bristles.

20. Basic color above wine red to cupreous red 21

Basic color above without any red 23

21. Middle band transverse at base and sinuately prolonged, or, rarely a short transverse bar 22

Middle band oblique, short ....*purpurea*

22. Middle band long, sinuate; other markings represented by dots ....*limbalis*

Middle band short, transverse; other marks indistinct or absent ....*limbalis* var. *transversa*

23. Color above dull greenish; thorax and elytra often tinged cupreous or bronze; middle band oblique, short ....*purpurea* var. *auduboni*

Color above black ....*purpurea* var. *nigerrima*

24. Markings reduced to an apical dot, often a short, transverse, narrow middle band ....*splendida*

Markings nearly complete; middle band long and sinuate ....*splendida* var. *cyanocephalata*

25. Markings reduced to dots or broken lines 26

Markings apparently complete 27

26. Form slender; each elytron with a row of large blue fovae near the suture; markings minute or absent ....*punctulata*

Form broad; elytral fovae not in a distinct row; markings broken into 12 or 14 dots (rarely only 10) ....*duodecimguttata*

27. Color above brown to greenish brown; marginal line present on elytra 28

Color above brilliant to blackish red; marginal line absent ....*fulgida*

28. Humeral lunule (-shaped; cheek with a few short, stiff hairs....*repanda*

Humeral lunule C-shaped with lower hook obliquely extended; cheek bare ....*hirticollis*

29. Elytra dark with white markings 30

Elytra predominantely white ....*lepida*

30. Markings complete; cheek pubescent 31

Markings absent or minute; color blackish; cheek bare ......*punctulata*

31. Color shining cupreous to green; elytra strongly and coarsely punctured, emarginate, forming an acute, prominent subapical tooth in the female, apex broadly rounded, truncate; male with feeble elytral emargination, apex rounded ....*cuprascens*

Color dull greenish or cupreous brown; elytra finely punctured, emarginate, forming an obtuse subapical tooth in the female; apex

of elytra in both male and female slightly prolonged, converging into the sutural spine .... *cuprascens* var. *macra*

CICINDELA FORMOSA var. GENEROSA Dej.

1831 *Cicindela formosa* var. *generosa* Dejean, Species gen. des. Coleop. coll. Dejean, vol. 5, p 231.
1834 *Cicindela formosa* var. *latecincta* Gould, Boston Jour. Nat. Hist., vol. 1, p 42.
1920 *Cicindela formosa* var. *generosa* Leng, Cat. Coleop. Amer., p 40.
1925 *Cicindela formosa* var. *fletcheri* Criddle, Can. Ent., vol. 57, p 127.
1930 *Cicindela formosa* race *generosa* Horn, Trans. Amer. Ent. Soc., vol. 56, p 79.

Length 14.5 mm. to 16 mm. Color above variable from dull red to brown with purplish tints, occasionally to greenish black; beneath metallic greenish blue. Elytra thickly punctate with shallow green impressions which tend to become indistinct in many of the western specimens such as those collected at Council Bluffs and Onawa, Iowa. Markings complete, connected by the marginal band, middle band sinuate, obtusely bent toward the apical lunule to terminate with a more or less hooked tip near the median suture. Pubescence erect, prominent on front, thoracic pleura, appendages and abdomen.

Distinguish from *formosa* by duller or darker brown, increased elytral punctation, deflexed middle band and more pubescent pronotal surface.

This distinct variation of *formosa* is largely limited to a warm, humid climate. It is common and easily recognized throughout most of Iowa. Near the western border, normally a more arid climate, specimens are lighter red in color, often slightly shining above; elytral punctures are less prominent and markings shortened.

Var. *generosa* is a sand inhabiting form, varying its preference greatly from high, dry dunes far removed from water to bars near rivers or creeks where the surface sand is scarcely dry.

Life cycle recorded by Shelford (1908): The eggs are laid during May and June in fresh sand. Larvae appear about July first to develop into the third stage by October first, hibernate and reappear in the spring. In the latter part of June and early July an oblique side cavity is directed from the burrow, about six or eight inches below the soil surface, to receive the prepupal stage. The imago emerges during late August or early September. The majority remain over winter in the pupal chamber while a few that come to the surface in September go into hibernation early in October. Larval life occupies twelve to thirteen months, adult life about ten months. Two years are required for one generation.

County records: Black Hawk, Boone, Bremer, Butler, Clinton, Delaware, Des Moines, Fayette, Hancock, Henry, Jackson, Jefferson, Linn, Louisa, Marion, Monona, Scott, Sioux, Story, Van Buren.

In addition to the above records Wickham (1911) records this species from Buchanan, Howard, Johnson and Pottawattamie Counties.

CICINDELA LENGI W. Horn

1848 *Cicindela venusta* LeConte, Ann. Lyc. Nat. Hist. N. Y., vol. 4, p 179. (Name preoccupied.)
1908 *Cicindela lengi* W. Horn, Duetsche Ent. Zeit., p 738.

1913 *Cicindela venusta* subsp. *versuta* Casey, Memoirs Coleop., vol. 4, p 24.
1913 *Cicindela venusta* subsp. *gracilenta* Casey, Memoris Coleop., vol. 4, p 25.
1920 *Cicindela lengi* Leng, Cat. Coleop. Amer., p 40.

Length 13 to 14.5 mm. Form slender. Color above, dull to brassy red; beneath, metallic green. Elytra granulate, feebly or not at all punctate; markings composed of a slightly enlarged and obliquely elongate humeral lunule, a sinuately deflexed middle band and complete apical lunule, all connected by the marginal band. Pubescence of front and prothorax prominent; ventral parts densely pilose.

In Nebraska *lengi, formosa* and var. *generosa* have been observed by the author, inhabiting the same sand hill, feeding among the sparse vegetation or in clear areas. *Lengi* immediately attracted attention to itself by the peculiar habit or raising the anterior part of the body when alarmed, apparently seeking a clearer view because this exercise was not included with normal activity and none were ovipositing.

Living in the sandy soil or pure sand of semi-arid localities, only a small portion of Iowa presents suitable habitat for *lengi;* however, it has been taken in Pottawattamie County by R. H. Wolcott.

The adults are abundant during May and early June, only a few occur in the fall.

County records: Pottawattamie, X, 14, 1907 (Wolcott). Nebr. Coll.

CICINDELA PURPUREA var. AUDUBONI Lec.

1845 *Cicindela auduboni* LeConte, Bost. Jour. Nat. Hist., vol. 5, p 207.
1883 *Cicindela purpura* var. *graminea* Schaupp, Bull. Brooklyn Ent. Soc., vol. 6, p 89.
1897 *Cicindela lauta* Casey, Ann. N. Y. Acad. Sci., vol. 9, p 296.
1913 *Cicindela purpurea* subsp. *ardelio* Casey, Memoirs Coleop., vol. 4, p 21.
1913 *Cicindela lauta* subsp. *franciscana* Casey, Memoirs Coleop., vol. 4, p 23.
1914 *Cicindela mirabilis* Casey, Memoirs Coleop., vol. 5, p 358.
1920 *Cicindela purpurea* var. *auduboni* Leng, Cat. Coleop. Amer., p 40.
1930 *Cicindela purpurea auduboni* Horn, Trans. Amer. Ent. Soc., vol. 56, p 80.
1932 *Cicindela purpurea* var. *auduboni* Nicolay & Weiss, Jour. N. Y. Ent. Soc., vol. 40, p 346.

Length 12 to 15 mm. Color above dull green, often suffused with cupreous reflections. Head and thorax greenish to cupreous bronze with green sutures and margins. Lateral plates of thorax cupreous, sutures green. Ventral parts metallic green, legs usually green, although bronzed on a few specimens. Elytra granulate, scarcely if at all punctate; markings reduced to a short oblique middle band, apical and small anteapical spot. In some specimens the vestige of a humeral or post humeral spot is visible. Pubescence of moderate density prevails on the front, thorax and legs; abdomen is sparsely haired.

This color variety found in Iowa is seldom confused with the true red *purpurea* although some specimens tend to retain a cupreous coloration. The records of *auduboni* are somewhat confused. LeConte (1845)

assigned this name to a green variety of *purpurea*. Later (1856) he applied the same name to the black variety which was used by subsequent authors until Leng (1918) discovered the error and named the black variety *nigerrima*. Consequently *graminea* of Schaupp (1883) is reduced to synonymy.

This variety prefers clay-like or loam soil of knolls, hills and uplands. The specimens observed by the author (April, 1934) were active on the small bare patches between clumps of grass on steep clay hills near Ansley, Nebraska. The species was also fund (October 7, 1936) on a little used road cutting through clay hills in Perkins County, Nebraska. The Iowa specimens were taken from territory closely resembling the Nebraska type. McCreary secured a specimen at the foot of a grassy slope not far from the Skunk River in Henry County.

Life history briefly summarized by Shelford (1908): The eggs are laid in May; larvae reach the last stage in August, hibernate, begin to feed again in April and pupate in July; the adults emerge in August, feed for a time, hibernate, come out in the spring to reach sexual maturity during April, lay eggs and die. The larval life lasts from 12 to 13 months and the adult 10 months. Two years are required for one generation.

County records: Henry, V, 1928 (McCreary); Linn, (Cedar Rapids, Iowa).

CICINDELA PURPUREA var. NIGERRIMA Leng

1857 *Cicindela purpurea* var. *auduboni* LeConte, Trans. Amer. Philos. Soc., ser. 2, vol. 11, p 37. (Name preoccupied.)
1918 *Cicindela purpurea* var. *nigerrima* Leng, Jour. N. Y. Ent. Soc., vol. 26, p 139.
1930 *Cicindela purpurea nigerrima* Horn, Trans. Amer. Ent. Soc., vol. 56, p 80.
1932 *Cicindela purpurea* var. *nigerrima* Nicolay & Weiss, Jour. N. Y. Ent. Soc., vol. 40, p 347.

Length 12 to 15 mm. Color above dull black; below slightly shining bluish black. Markings as in *purpura* and var. *auduboni*. Pubescence more conspicuous than on *purpurea*.

This variety is easily distinguished by its color; however, retention of typical *purpurea* shape, markings and habit denote its close relationship. Collected rarely and sporadically it may be only a mutation or abnormality rather than a true variety.

The *auduboni* Lec. reported from northwest Iowa by Wickham (1911) is undoubtedly *nigerrima* Leng.

Habits of *nigerrima* are similar to *auduboni*. The specimens recorded were taken with var. *auduboni* and from situations typical of *purpurea*.

County records: Woodbury, (Wickham, 1911).

CICINDELA SPLENDIDA Hentz

1830 *Cicindela splendida* Hentz, Trans. Amer. Philos. Soc., ser. 2, vol. 6, p 254.
1834 *Cicindela discus* Klug, Jahrbucher der insectenkunde, vol. 1, p 23.
1884 *Cicindela splendida* Hentz, Bull. Brooklyn Ent. Soc., vol. 6, p 112 (reprint of 1830).
1920 *Cicindela splendida* Leng, Cat. Coleop. Amer., p 40.

1930 *Cicindela purpurea* race *splendida* Horn, Trans. Amer. Ent. Soc., vol. 56, p 80.

1932 *Cicindela splendida* Nicolay & Weiss, Jour. N. Y. Ent. Soc., vol. 40, p 350.

Length 12.5 to 15 mm. Characterized by the metallic greenish blue or french blue color of the head and thorax in contrast to the elytra which vary from dark to bright red, with a brassy sheen evident on some of the western specimens. The markings consist of a short, transverse, marginal remnant of the middle band, the apical lunule, frequently an anteapical spot and rarely a humeral spot. Elytra granulate, scarcely punctate. Front, thorax and appendages moderately pubescent.

A few specimens taken in Iowa were collected from clay canyons in the northeast section inhabited by *limbalis,* its variety *transversa* and *splendida* var. *cyanocephalata.* Local areas may be found (Lincoln, Nebraska) dominated by *splendida* (the variety *cyanocephalata* most numerous). However, in Iowa only a few specimens are found among many *limbalis.*

The month of May is the most opportune period for collecting this species. Frequently the use of a net is prohibited by the uneven ground. With this species unusual success may be enjoyed by employing this unique method: Secure a dried sunflower stem about 8 or 10 feet long which has several small branches at the tip. Thus equipped walk slowly through the canyon or area inhabited by *splendida.* When one is sighted briskly slap the ground within a few inches of the insect, creating a continuous rattle that usually paralizes the beetle momentarily. An active collector can secure his specimen during the three or four seconds required for the insect to "collect its wits." However, an assistant is a valuable asset.

County records: Dubuque, V, 1934 (Boughton), V, 1936 (Eckhoff).

Wickham (1911) records this species from Pottawattamie and Scott Counties.

CICINDELA SPLENDIDA var. CYANOCEPHALATA n. var.

1911 *Cicindela limbalis* var. *amoena* Harris, N. Amer. Cicindelidae in Harris Coll., p. 8. (Name preoccupied.)

1928 *Cicindela purpurea* subsp. *splendida* form *cyanocephala* Varas, Rev. Chil. Hist. Nat., vol. 32, p 239. (Name preoccupied.)

1930 *Cicindela purpurea* race *cyanocephala* Horn, Trans. Amer. Ent. Soc., vol. 56, p 80.

1931 *Cicindela limbalis* var. *cycanocephala* Leng, Second Sup. Cat. Coleop. Amer., p 9.

1932 *Cicindela splendida* var. *cyanocephala* Nicolay and Weiss, Jour. N. Y. Ent. Soc., vol. 40, p 351.

Length 12.5 to 15 mm. Head and thorax greenish blue or french blue; elytral color similar to *spendida* but with prominent white markings of the *limbalis* type, being composed of a sinuate middle band, anteapical and apical spots. Humeral and posthumeral spots usually present, although some specimens lack one or both.

Specimens of this type were formerly included with *splendida* (Leng 1902) until Varas (1928) applied the name *Cicindela purpurea* subsp. *splendida cyanocephala.* Unfortunately *cyanocephala* Varas is pre-

occupied (*Cicindela cyanocephala* Fabr., 1798 Fabricius, Supplementum Entomologiae Systema, Hafniae, p 60) which necessitates a new name.

They occur with *splendida* often outnumbering the typical parent species. *Limbalis*, the variety *transversa* and *splendida* var. *cyanocephalata* have been observed inhabiting the same canyons of clay and loam hills near Maquoketa Park in Jackson County.

Specimens of *limbalis* mating with var. *cyanocephalata* have been taken and now repose in the Nebraska collection.

The close relationship of the *limbalis* group has been suggested in order to clarify the scope of the varietal names rather than belittle their value. Some varietal names have been retained to consign specimens of dubious rank to their parent species rather than to establish a number of races.

County records: Jackson, V, 1936 (Eckhoff).

CICINDELA LIMBALIS Klug

1834 *Cicindela limbalis* Klug, Jahrbucher der insectenkunde, p 29.

1848 *Cicindela limbalis* var. *amoena* LeConte, Ann. Lyc. Nat. Hist. N. Y., vol. 4, p 177.

1857 *Cicindela splendida* LeConte, Trans. Amer. Philos. Soc., ser. 2, vol. 11, p 36. (Name cited in error.)

1913 *Cicindela limbalis* subsp. *awemeana* Casey, Memoirs Coleop., vol. 4, p 23.

1913 *Cicindela limbalis* subsp. *eldorensis* Casey, Memoirs Coleop., vol. 4, p 23.

1920 *Cicindela limbalis* Leng, Cat. Coleop. Amer., p 40.

1928 *Cicindela limbalis* var. *militaris* Varas, Rev. Chil. Hist. Nat., vol. 32, p 242.

1930 *Cicindela purpurea* race *limbalis* Horn, Trans. Amer. Ent. Soc., vol. 56, p 80.

Length 13 to 15 mm. Color above variable in shade and intensity. Iowa specimens display colors from bright red to dull reddish brown with cupreous to wine colored intermediates. Sutures and margins bright metallic green or blue. Color below metallic bluish green with cupreous reflections. Front thorax and appendages moderately pubescent; abdomen sparsely so. The elytra are granulate, feebly punctate; markings usually prominent, composed of humeral, posthumeral dots (sometimes reduced or entirely absent), apical, anteapical spots (seldom confluent to form complete acipal lunule) and a sinuate middle band directed transversely from the margin to the middle of the elytron before being deflexed at an obtuse angle towards the median suture.

This species is restricted to the clayey soils constituting a large part of the rough, hilly land within the state. The adults are collected in steep gullies and small canyons defacing a treeless hillside, or frequently found among vegetation near the brink. The adults appear early in April, are most abundant during May and early June; a few stragglers occur as late as July.

County records: Appanoose, Davis, Des Moines, Dickinson, Dubuque, Henry, Jackson, Jefferson, Johnson, Louisa, Page, Poweshiek, Scott, Story, Van Buren, Wayne.

In addition to the above records Wickham (1911) records this species from Cherokee, Franklin, Howard and Pottawattamie Counties.

CICINDELA LIMBALIS var. TRANSVERSA Leng

1902 *Cicindela purpurea* var. *transversa* Leng, Trans. Amer. Ent. Soc., vol. 28, p 131.
1911 *Cicindela limbalis* var. *transversa* Harris, N. Amer. Cicindelidae in Harris Coll., p 7.
1920 *Cicindela splendida* var. *transversa* Leng, Cat. Coleop. Amer., p 40.
1930 *Cicindela purpurea* race *splendida* var. *transversa* Horn, Trans. Amer. Ent. Soc., vol. 56, p 80.
1932 *Cicindela limbalis* var. *transversa* Nicolay & Weiss, Jour. of N. Y. Ent. Soc., vol. 40, p 348.

Length 13 to 15 mm. Form and color similar to *limbalis* but with a tendency toward the brighter color; head and thorax frequently exhibit a brassy luster. The chief differentiating character is the reduction of the middle band to a short transverse bar. The humeral, posthumeral and anteapical dots are usually less distinct than on *limbalis,* often entirely absent.

This variety is a very slight departure from the parent species *limbalis* but is retained by Nicolay and Weiss (1932) because it represents an intermediate or connecting link between *limbalis* and *splendida.*

County records: Jackson, V, 1936 (Eckhoff); Jefferson, V, 1931 (Pierson); Johnson, III, (Wickham).

CINCINDELA DUODECIMGUTTATA Dej.

1825 *Cicindela duodecimguttata* Dejean, species gen. Coleop. coll. Dejean, vol. 1, p 73.
1837 *Cicindela proteus* Kirby, Insects in Richardson's Fauna Boreali-Americana, vol. 4, p 9.
1913 *Cicindela bucolica* Casey, Memoirs Coleop., vol. 4, p 28.
1916 *Cicindela hudsonica* Casey, Memoirs Coleop., vol. 7, p 29.
1920 *Cicindela repanda edmontonensis* Carr, Can. Ent., vol. 52, p 218.
1920 *Cicindela duodecimguttata* Leng, Cat. Coleop. Amer., p 40.
1930 *Cicindela duodecimguttata* Horn, Trans. Amer. Ent. Soc., vol. 56, p 80.

Length 12 to 14 mm. Dorsum dull dark brown, head and pronotum faintly bronzed; color beneath dark metallic green or greenish blue, thoracic pleura cupreous or bronzed. Elytra densely punctate with greenish blue impressions; marked by humeral and posthumeral spots which occasionally form a complete lunule, a narrow, usually interrupted middle band, anteapical and apical spots which are rarely connected to form a complete lunule. Prothorax nearly margined, only slightly convex; pronotum about one millimeter broader than long, distinctly broadened and angulate anteriorly. Pubescence erect, usually dense on thoracic pleura and appendages; head, lateral borders of pronotum and venter inconspicuously hairy.

Distinguished from *repanda* by larger prothorax, usually reduced markings and darker color.

Duodecimguttata is distributed over the entire state, but restricted to a special habitat. Specimens have been found only on wet loam or clay soil. The ideal habitat is the steep sides of gullies or ravines in which a small trickle of water flows over the hard, smooth clay. The species frequents many of the pond, creek and lake shores which contain abundant

humus. The adults are numerous in May; a few occur throughout the summer.

County records: Appanoose, Cerro Gordo, Davis, Des Moines, Dubuque, Fremont, Harrison, Henry, Jackson, Jefferson, Keokuk, Mills, Mitchell, Monroe, Montgomery, Page, Poweshiek, Story, Van Buren, Washington, Wayne.

Wickham (1911) records this species from Howard, Johnson and Linn Counties. Casey (1913) records this species from Lee County.

CICINDELA REPANDA Dej.

1825 *Cicindela repanda* Dejean, Species gen. Coleop. coll. Dejean, vol. 1, p 74.
1834 *Cicindela hirticollis* Gould, Boston Jour. Nat. Hist., vol. 1, p 49.
1857 *Cicindela baltimorensis* LeConte, Trans. Amer. Philos. Soc., ser. 2, vol. 11, p 43.
1897 *Cicindela unijuncta* Casey, Ann. N. Y. Acad. Sci., vol. 9, p 299.
1920 *Cicindela repanda* Leng, Cat. Coleop. Amer., p 40.
1930 *Cicindela duodecimguttata* race *repanda* Horn, Trans. Amer. Ent. Soc., vol. 56, p 81.

Length 10.5 to 13 mm. Dorsum light brown to greenish brown with greenish or cupreous bronze reflections on head and pronotum; beneath bright metallic green; thoracic pleura and portions of the legs cupreous bronze. Elytra densely punctate with shallow green or bluish impressions; markings complete, composed of C-shaped humeral lunule, sinuate middle band, distinct marginal line occasionally produced anteriorly to connect the humeral lunule, and a complete apical lunule. Pubescence erect, usually dense on thoracic pleura and appendages; moderately dense on front, lateral borders of pronotum and venter.

Distinguished from *duodecimguttata* by complete markings, narrow convex prothorax and usually brighter color; from *hirticollis* by the shorter humeral lunule, setae on cheek and broadly rounded elytral apex.

A hardy, very active species, *repanda* is common in the vicinity of nearly all damp sandy situations; abundant on the banks and and bars of all Iowa rivers, streams and lakes. Occasionally individuals are found associating with *duodecimguttata* but they do not flourish in that habitat.

Somewhat gregarious, many adults have been observed, within a small area on the moist sandy shore of a stream, busily catching minute insects or chasing each other. They pass the night and cool days in short (one inch) oblique tunnels dug in the fine, dry sand a short distance from the water's edge, reappearing when warmer temperature prevails.

County records: Appanoose, Benton, Black Hawk, Boone, Bremer, Buena Vista, Butler, Cass, Clarke, Clay, Clinton, Davis, Des Moines, Dickinson, Dubuque, Emmet, Fayette, Henry, Iowa, Jackson, Jefferson, Johnson, Linn, Louisa, Lyon, Madison, Mitchell, Monona, Monroe, Montgomery, Page, Plymouth, Polk, Pottawattamie, Scott, Sioux, Story, Union, Van Buren, Wapello, Warren, Washington, Wayne, Webster, Woodbury.

CICINDELA HIRTICOLLIS Say

1817 *Cicindela hirticollis* Say, Jour. Acad. Nat. Sci. Phila., vol. 1, p 20.
1826 *Cicindela albohirta* Dejean, Species gen. Coleop. coll. Dejean, vol. 2, p 425.

1836 *Cicindela unita* Kollar, Ann. d. Wien. Mus., vol. 2, p 330.
1920 *Cicindela dejeani* LeConte (in litt.) Leng, Cat. Coleop. Amer., p 40.
1920 *Cicindela humeralis* LeConte (in litt.) Leng, Cat. Coleop. Amer., p 40.
1920 *Cicindela hydropica* Dupuis (in litt.) Leng, Cat. Coleop. Amer., p 40.
1920 *Cicindela hirticollis* Leng, Cat. Coleop. Amer., p. 40.
1930 *Cicindela hirticollis* Horn, Trans. Amer. Ent. Soc., vol. 56, p 81.

Length 13 to 15.5 mm. Dorsum brownish bronze, often with greenish reflections; color beneath bright green with brilliant cupreous reflections on thoracic pleura. Elytra densely punctate with large, bluish green impressions; markings complete, prominent. Pronotum slightly convex, nearly quadrate, the angles rounded. Pubescence erect, sparse on the front and pronotal surface, dense on thoracic pleura and appendages; outer edges of venter moderately hairy.

Distinguished from *repanda* by long slender mandibles, bare cheeks, larger area of pronotal pubescence, slight apical prolongation of elytra and characteristic projection of the humeral lunule toward the median suture.

Hirticollis apparently is a strictly fluviatile species. The author has collected it only from the banks of the Des Moines, Iowa, Mississippi, Missouri and Wapsipinicon Rivers. Numerous during a period of subnormal water levels, the beetles frequent the silt-covered bars at the water's edge. The slime, mud and organic matter exposed by receding water is attractive to countless numbers of tiny insects and consequently numerous predators of which *hirticollis* and *cuprascens* var. *macra* constitute the majority.

Feeding *hirticollis* have been observed to cover forty to sixty feet of shoreline without straying more than a foot from the water; often wading through or into puddles three or four millimeters deep.

The adults appear late in June or early July; continue in evidence until late August.

County records: Clinton, VII, 1936 (Eckhoff); Dickinson, VI, 1932 (Galer), VI, 1932 (Moore); Henry, VIII, 1930 (Parks); Louisa, VIII, 1936 (H. M. Harris); Monona, VIII, 1932 (A. M. Pearson), VII, 1936 (Eckhoff); Scott, VII, 1936 (Eckhoff); Van Buren, VII, 1936 (Eckhoff).

Wickham (1911) records this species from Black Hawk, Johnson and Pottawattamie Counties.

CICINDELA TRANQUEBARICA Hbst.

1806 *Cicindela tranquebarica* Herbst, Natursystem etc., vol. 10, p 178.
1818 *Cicindela vulgaris* Say, Trans. Amer. Philos. Soc., vol. 1, p 409.
1825 *Cicindela obliquata* Dejean, Species gen. Coleop. coll. Dejean, vol. 1, p 72.
1910 *Cicindela tranquebarica* var. *minor* Leng, Jour. N. Y. Ent. Soc., vol. 18, p 80.
1911 *Cicindela tranqueberica* var. *vulgaris-minor* Harris, N. Amer. Cicindelidae in Harris Coll., p 18.
1913 *Cicindela crinifrons* Casey, Memoirs Coleop., vol. 4, p 26.
1914 *Cicindela wichitana* Casey, Memoirs Coleop., vol. 5, p 21.
1914 *Cicindela tranquebarica* subsp. *turbulenta* Casey, Memoirs Coleop., vol. 5, p 25.

1920 *Cicindela tranquebarica* Leng, Cat. Coleop. Amer., p 40.
1930 *Cicindela tranquebarica* Horn, Trans. Amer. Ent. Soc., vol. 56, p 81.

Length 13 to 16 mm. Color above dark brown, occasionally greenish, or in some specimens opaque black; head, thorax and legs metallic cupreous bronze; sutures blue or green and abdomen metallic green. Front, thoracic pleura and appendages conspicuously hairy. Elytra somewhat irregularly and coarsely punctate by shallow green or blue impressions with a few dispersed fovae on basal third. Distinct markings of moderate size include an obliquely elongate humeral lunule curving slightly forward toward the suture, or, produced posteriorly to approach the obtuse angle of the middle band, a rather narrow middle band which is only slightly if at all extended at the margin, nearly transverse before deflecting apically at an obtuse angle to terminate in a broad hook near the median suture and a complete apical lunule with the anterior portion inflexed.

This species appears quite widely distributed within the state, but is not plentiful in any locality. Apparently the local distribution of the species is influenced by available soil moisture. The females select only situations with a fairly constant moisture supply in which to deposit eggs. These moisture conditions differ widely due to the large temperature range within the state. Although no notable color variation of *tranquebarica* has been observed in Iowa the specimens exhibit considerable differences in the type of marking, evidently brought about by temperature fluctuations during development. Consideration of these facts and the susceptibility of the species to environmental influence should halt any attempt to name varieties of *tranquebarica* on the basis of color pattern.

Life cycle stated by Shelford (1908): The eggs are laid in May. Hatching in two weeks the larvae reach the last stage in August, hibernate, begin to feed in April and pupate in July. The adults emerge in August, feed for a time, hibernate and come out in the spring, mature sexually during April, oviposit during May and die.

Larval life 12 to 13 months, adult life 10 months; 2 years required to complete one generation.

County records: Boone, V, 1936 (Eckhoff); Bremer, V, 1936 (Eckhoff); Fremont, IV, 1932 (Clark), IV, 1935 (Janes); Lee, V, 1932 (Kagy); Monona, VII, 1932 (Galer); Osceola, VI, 1932 (Galer); Page, VIII, 1932 (Loudon); Story, (1892 Exp. Sta.).

Wickham (1911) records this species from Johnson and Pottawattamie Counties.

CICINDELA SCUTELLARIS var. LECONTEI Hald.

1853 *Cicindela scutellaris* var. *lecontei* Haldeman, Proc. Acad. Nat. Sci. Phila., vol. 6, p 361.
1920 *Cicindela scutellaris* var. *lecontei* Leng, Cat. Coleop. Amer., p 41.
1930 *Cicindela scutellaris modesta* Horn, Trans. Amer. Ent. Soc., vol. 56, p 83.

Length 10.5 to 13 mm. Color above typically a cupreous red; however, every intermediate shade to a coppery or greenish bronze is represented; beneath, head and thorax metallic cupreous or bronze; abdomen green with cupreous reflections. Elytra usually impunctate frequently with a row of inconspicuous fovae near the suture; occasionally a specimen exhibits feeble punctation. Markings, although variable, commonly con-

sist of the posthumeral spot, triangular marginal spot and complete apical lunule. Frequently specimens display a complete humeral lunule becoming confluent with the marginal spot; rarely do the markings form an intact marginal band. Frontal pubescence prominent on the male; very sparse on the female, sometimes only the strong orbital bristles are present.

This species inhabits the dry sandy soil, flourishing in areas of pure, fine blow-sand supporting sparse growth of vegetation seldom visited by man or animal. In Story County this type of soil is negligible, but a few specimens have been taken from dry gravel pits and from high dry sand bars deposited by a stream at flood stage. Abundant numbers of *lecontei* occurred in Backbone Park (Delaware County) during May, feeding among sparse vegetation bordering dry, shallow, extremely sandy washes; very few could be found July first but they were numerous October first. In northwestern Clinton County (May 16, 1936) both *lecontei* and *generosa* were found frequenting a steep sandy hill partially covered with native grasses.

Shelford (1908) states that the production of one generation occupies two years. The eggs are laid during May in dry sand containing little humus. The larvae reach the last stage in August or September, hibernate, begin to feed again in April and pupate in July. The adults emerge in August or September, feed for a time, hibernate until April, reappear to mate and oviposit during May. The larval life occupies 12 or 13 months, adult life about 10 months.

Apparently the extreme heat or drought of 1936 inhibited pupal development as adults failed to appear until late September and early October.

County records: Black Hawk, Boone, Bremer, Butler, Clinton, Delaware, Fayette, Fremont, Henry, Lee, Linn, Louisa, Muscatine, Story.

Wickham (1911) records this species from Buchanan, Clayton, Howard, Johnson and Pottawattamie Counties.

CICINDELA SEXGUTTATA Fabr.

1775 *Cicindela sexguttata* Fabricius, Systema Entomologica, p 226.
1799 *Cicindela varians* Ljungh, Vetensk. Acad. Nya. Handl., vol. 20, p 147.
1854 *Cicindela guttata* Emmons, The Nat. Hist. of Agr. of N. Y., vol. 5, p 35.
1903 *Cicindela quadriguttata* Davis, Ent. News, vol. 14, p 270.
1909 *Cicindela levettei* Casey, Can. Ent., vol. 41, p 270.
1920 *Cicindela sexguttata* Leng, Cat. Coleop. Amer., p 41.
1920 *Cicindela sexguttata* var. *levettei* Leng, Cat. Coleop. Amer., p 41.
1930 *Cicindela sexguttata* Horn, Trans. Amer. Ent. Soc., vol. 56, p 83.

Length 11.5 to 14 mm. Color above commonly shining green, often with bluish reflections; below, metallic greenish to violaceous. Elytra somewhat granulate, coarsely punctate; marginal, anteapical, apical and frequently a discal spot constitute the markings of each elytron. Front glabrous, pubescence of ventral parts erect, feeble and sparse.

Typical *sexguttata* is more prevalent in the southeastern part of the state while the variety *tridens* is the member most frequently collected in the central and northwestern territory.

This species inhabits clear woods, usually of oak, ash and hickory

which constitute the majority of Iowa groves and forests. Adults were numerous (May 15, 1936) in the upland woods bordering a small tributary of the Mississippi River (Des Moines County). Here the author observed individuals congregated in the clear sunny spots on the banks of the stream at the edge of the woods. Seemingly less alert than most species the sudden appearance of an intruder caused hasty, disorganized retreat. Some individuals quietly crept into concealment under nearby rubbish while others after running about excitedly made a short flight, often hiding to elude further pursuit. None could be found outside of the woods; however, specimens are collected near ditches or gullies in fields and pastures. The author found one specimen (Johnson County, VII, 1935) trapped in a post hole dug in connection with a chinch bug barrier.

In Maquoketa Park (Jackson County) on July 13, 1936, a few *sexguttata* and variety *tridens* were found together among the sparse vegetation on the banks of a small, dry, sandy stream bed.

Life history from Shelford (1908): The eggs are laid during June or early July in shaded sand or clay containing humus. The majority of the larvae pass winter in the third stage, few in the second stage. The pupal stage is reached by July the following summer; however, very few imagoes leave the pupal chamber until spring rains soften the soil. Any adults emerging in the fall hibernate under logs or leaves, but do not dig into the earth.

County records: Bremer, Clarke, Davis, Des Moines, Dickinson, Grundy, Henry, Jackson, Jefferson, Johnson, Lee, Linn, Louisa, Mahaska, Monroe, Muscatine, Page, Scott, Van Buren, Washington.

CICINDELA SEXGUTTATA var. TRIDENS Csy.

1909 *Cicindela levettei* var. *tridens* Casey, Can. Ent., vol. 41, p 271.
1920 *Cicindela sexguttata* var. *levettei* Leng, Cat. Coleop. Amer., p 41.
1930 *Cicindela sexguttata tridens* Horn, Trans. Amer. Ent. Soc., vol. 56, p 83.

Length 11.5 to 14 mm. Color above shining green or with purplish reflections. Form, pubescence and elytral punctation similar to *sexguttata* from which it is distinguished only by the reduced color pattern. The majority of specimens are immaculate although retention of a small marginal spot frequently augmented by remnants of an apical spot is not uncommon.

Among the *Cicindela* in the Iowa State College student collections *sexguttata* is represented only by the immaculate form, many of which bear incorrect determination labels. Evidently the student does not associate this form with a "six spotted" species. Rarely are typical *sexguttata* taken in the northwestern half of the state, therefore, the author has retained the varietal name *tridens* Csy. to promote more accurate association with the true species.

Leng (1920) graciously recognized *levettei* Csy. as a variety of *sexguttata* and listed *tridens* as synonymous to *levettei*. Horn (1930) quite correctly relegated *levettei* to synonymy and considered *tridens* to be a race of *sexguttata*.

The author has been unable to distinguish *levettei* from *sexguttata*. However, *tridens* can be separated by the characteristic reduction of the markings.

The variety occurs throughout the state. It is decidedly outnumbered by true *sexguttata* in the southern portion but almost exclusively dominates the northwestern territory.

The adults delight in sparsely wooded areas that permit considerable growth of annual vegetation, where they may feed in the open sunny spots or rest in the adjacent shade. They exhibit the same furtive habits noted for *sexguttata*. Early spring collectors, while combing the woods near the Iowa State College campus for insects, occasionally find a *tridens* hibernating under a log or the loose bark of a stump or tree. *Tridens* was numerous on May 16, 1936, in company with a few *sexguttata*, along the grassy banks of a dry stream bed cutting through Maquoketa Park (Jackson County). The population was greatly reduced by July 13, 1936. Among the adult *sexguttata* observed in Des Moines County May 15, 1936, near a small stream bordered by woods, relatively few exhibited reduced markings; thus it seems evident some environmental factor must influence the deposition of elytral pigment.

The life cycle does not differ from that of *sexguttata*.

County records: Adair, Appanoose, Boone, Buchanan, Cedar, Clarke, Clayton, Clinton, Delaware, Des Moines, Dickinson, Harrison, Henry, Jackson, Jasper, Jones, Johnson, Kossuth, Louisa, Madison, Marshall, Page, Story, Van Buren.

CICINDELA SEXGUTTATA var. VIOLACEAE Fabr.

1801 *Cicindela violaceae* Fabricius, Systema Eleutheratorium, p 232.
1920 *Cicindela sexguttata* var. *violaceae* Leng, Cat. Coleop. Amer., p 41.
1929 *Cicindela Kansanus* Knaus, Jour. Kan. Ent. Soc., vol. 2, p 24.
1930 *Cicindela sexguttata violaceae* Horn, Trans. Amer. Ent. Soc., vol. 56, p 83.

Length 12 to 13.5 mm. Color of entire body, except antennae and mouth parts, rich, deep violet hue with no hint of greenish reflections. Form, pubescence and elytral punctation similar to *sexguttata*. The Iowa form is usually immaculate; however, a marginal or apical spot and occasionally both may be present.

This color variation of *sexguttata* is only collected occasionally and to the author it represents a form in which physiological conditions during prepupal and pupal development were such that the abnormal coloration was produced.

Violaceae frequents the dry channels of creeks and gullies or stream banks of wooded areas in company with *sexguttata* and variety *tridens*.

County records: Story, VI, 5, 1931 (H. M. Harris), 1 specimen (Ames), old label.

Wickham (1911) records this species from Des Moines County and Knaus (1929) records this species from Johnson County.

CICINDELA PUNCTULATA Oliv.

1790 *Cicindela punctulata* Olivier, Entomologie, vol. 2, p 70.
1798 *Cicindela micans* Fabricius, Suppl. Ent. Syst., p 61.
1806 *Cicindela obscura* Melsheimer, Cat. Insects (beetles) of Penn., p 46.
1837 *Cicindela jensoni* Gistel, Systema Insectorum, vol. 1, p 55.
1909 *Cicindela boulderensis* Casey, Can. Ent., vol. 41, p 271.
1916 *Cicindela prolixa* Casey, Memoirs Coleop., vol. 7, p 33.

1916 *Cicindela fontinaria* Casey, Memoirs Coleop., vol. 7, p 33.
1920 *Cicindela punctulata* Leng, Cat. Coleop. Amer., p 41.
1930 *Cicindela punctulata* Horn, Trans. Amer. Ent. Soc., vol. 56, p 84.

Length 11 to 13 mm. Form slender; thorax more or less cylindrical; legs long. Color above mostly black, often with brownish bronze reflections on the elytra; head and thorax more metallic; sutures and margins often greenish blue. Color below dark metallic blue, the prosternum and met-episterna tinged cupreous or bronze. Elytra coarsely punctate, finely serrulate at apex; a row of prominent green or blue fovae extend from the base to the apex of each elytron, flanked by a double row extending a short distance back of the humeral angle. Markings, other than the constant apical lunule, variable; at most including an interrupted humeral lunule, interrupted marginal line and a middle band represented by three dots. Minimum markings consist of the slender apical lunule and remnants of a marginal line. Pubescence short, often appearing decumbent, sparsely distributed on ventral parts; absent from the head.

Punctulata having greater diversity of habits than other members of the family is also the most common species in Iowa. A strong flier and voracious feeder it occurs in a variety of situations. It has been collected from wet sand at the river's edge, resting in the shade of sticks, stones and vegetation when the heat becomes severe, or when opportunity affords, large numbers crawl into the cracks made by the baking of mud from which the water has receded. It is frequently found in fields of alfalfa, corn, small grains; pastures, gardens, lawns; along hard paths and dusty roads. Occasionally they are found in sparse woods and on dry sandy dunes in company with *lecontei* and *generosa.*

Aside from feeding throughout the day, large numbers are attracted to lights at night. In cities (Cedar Rapids, Des Moines, Ottumwa and Sioux City) adults have been observed running along the sidewalks preying upon other small nocturnal insects. Some were in evidence as late as eight o'clock the following morning before they sought a more suitable hunting ground.

Balduf (1925) observed a specimen of *punctulata* in the shade of tall corn devour nine nymphs and one adult chinch bug during a 26-minute period within an area six inches in diameter.

In spite of consuming rather unsavory foods *punctulata* emits a pleasant, fruity odor when first captured.

The adults appear during late June, are abundant throughout July and August, subside in September and disappear completely by October.

County records: Allamakee, Audubon, Boone, Bremer, Buchanan, Buena Vista, Calhoun, Cass, Cerro Gordo, Cherokee, Chickasaw, Clarke, Clay, Clayton, Clinton, Dallis, Davis, Decatur, Delaware, Des Moines, Dickinson, Harrison, Henry, Iowa, Jackson, Jasper, Jefferson, Johnson, Lee, Linn, Louisa, Madison, Mahaska, Mills, Monona, Muscatine, O'Brien, Osceola, Page, Plymouth, Polk, Scott, Sioux, Story, Union, Van Buren, Wapello, Webster, Woodbury, Wright.

CICINDELA CELERIPES Lec.

1848 *Cicindela celeripes* LeConte, Ann. Lyc. Nat. Hist. N. Y., vol. 4, p 183.
1920 *Cicindela celeripes* Leng, Cat. Coleop. Amer., p 42.

Length 7 to 9 mm. Color brownish bronze above, head and prothorax

greenish bronze; ventral surface greenish or brown, trochanters and palpi pale, legs greenish. Elytra reduced at the base, forming rounded, indistinct humeral angles; coarsely punctate with green punctures and sparsely clothed with short decumbent hairs. Each elytron rounded at the apex and bearing a prominent sutural spine. The markings are variable, generally represented by a posthumeral and discal dot in addition to a marginal line and complete apical lunule; however, they may be reduced to marginal and apical dots.

The species was collected by Wickham in 1898 among scattered grass clumps on the prairie bluffs near Council Bluffs, Iowa, and frequenting the upper end of a deep gulley formed in the clay soil near Iowa City. Numerous specimens were secured by Nebraska collectors during July of the year 1905, 1906, 1908 and 1912 from hilly prairie land near the Missouri River.

Although unable to fly, the agility of the adults is often discouraging to a collector.

County records: Pottawattamie, VI, 1905 (Shoemaker) Nebr. collection.

CICINDELA CURSITANS Lec.

1857 *Cicindela cursitans* LeConte, Trans. Amer. Philos. Soc., ser. 2, vol. 11, p 60.

1920 *Cicindela cursitans* Leng, Cat. Coleop. Amer., p 42.

Length 7 to 9 mm. Closely resembles *celeripes* in color and form. However, the elytra are glabrous, finely punctate, with broader markings and a less prominent sutural spine. Legs are light brown or testaceous.

Habits apparently the same as *celeripes*. Wickham (1898) reports one specimen taken on a bank of the Iowa River near Iowa City, Iowa. A few were taken during June near Council Bluffs, Iowa, by F. H. Shoemaker.

County records: Pottawattamie, VI, 1905 (Shoemaker) Nebr. collection.

CICINDELA UNIPUNCTATA Fabr.

1775 *Cicindela unipunctata* Fabricius, Syst. Ent., p 225.

1920 *Cicindela obsoleta* Dejean (in litt.) Leng, Cat. Coleop. Amer., p 42.

1920 *Cicindela unipunctata* Leng, Cat. Coleop. Amer., p 42.

1930 *Cicindela unipunctata* Horn, Trans. Amer. Ent. Soc., vol. 56, p 85.

Length 15 to 18 mm. Color above opaque, mossy brown; beneath dark metallic blue. Elytra flattened to an unusual extent, roughened by irregular depressions, dansely punctured with coarse,green impressions and dispersed fovae; markings confined to a single small marginal spot. Pronotum with a feeble fringe of hair; thorax and abdomen glabrous. Head strongly strigose; labrum tridentate, somewhat elongate.

This species is rarely encountered, probably escaping notice because of its protective coloration and cautious habits. Contrary to the majority of *Cicindela* it shuns open, sunny situations but frequents dense woods either singly or in pairs, not in company with other species.

Mr. Kongable of Iowa Wesleyan College collected a specimen (May 21, 1936) near the Skunk River in Henry County. He secured the beetle in a sweeping net about mid-afternoon on a clear day while collecting in a heavily wooded hollow thickly overgrown with weeds.

Wickham (1898) found one specimen in Cedar County on a dark, rainy day late in May while collecting along the Cedar River bottoms. The insect ran across a path into some dead leaves nearby from whence it was readily captured.

Records indicate the adults are present from May to July.

County records: Henry, VII, 23, 1924 (Jaques), VII, 27, 1929 (McCreary), V, 21, 1936 (Kongable), V, 25, 1936 (Gardner); Louisa, VI, 7, 1935 (Warren).

CICINDELA CUPRASCENS Lec.

1825 *Cicindela cuprascens* LeConte, Proc. Acad. Nat. Sci. Phila., vol. 6, p 65.
1913 *Cicindela cuprascens* subsp. *amnicola* Casey, Memoirs Coleop., vol. 4, p 37.
1920 *Cicindela cuprascens* Leng, Cat. Coleop. Amer., p 42.
1930 *Cicindela cuprascens* Horn, Trans. Amer. Ent. Soc., vol. 56, p 86.

Length 11 to 13 mm. Dorsum shining cupreous or greenish bronze; beneath bright metallic green, palpi pale, trochanters rufous. Elytra coarsely and densely punctate; in the female emarginate externally at apex to form a prominent, acute subapical tooth, tips rounded; in the male obliquely sinuate at apex, tips obtuse. Markings complete, connected by marginal band, middle band often slender and tortuous; small spot at base of each elytron frequently connected to anterior portion of humeral lunule. Pubescence short, decumbent, moderately dense on front, occiput and pronotum; very dense on cheek, thorax and sides of venter.

Distinguished from *macra* by brighter, more shining colors; coarser and deeper elytral punctures and the more prominent, acute subapical tooth and rounded tips of female elytra or more obtusely rounded tips of the male elytra.

This species frequents the wet sandy banks of the Missouri River and is rarely found elsewhere. Adults were abundant near the river at Onawa, Iowa, July 29, 1936, and were collected without difficulty on the packed wet sand.

Records and observations indicate that adults appear in late June or early July; mature sexually, reproduce and pass away by August 15.

County records: Bremer, VII, 1923 (B. G. J.); Monona, VIII, 1932 (Pearson), VII, 1936 (Eckhoff); Pottawattamie, V, 1930 (?), VII, 1934 (Knutson); Story (Ames, Iowa), old label, very dubious record; Wapello, VIII, 1931 (Moore); Woodbury, VII, 1936 (Eckhoff).

CICINDELA CUPRASCENS var. MACRA Lec.

1857 *Cicindela cuprascens* var. *macra* LeConte, Trans. Amer. Philos. Soc., ser. 2, vol. 11, p 50.
1913 *Cicindela macra* subsp. *mercurialis* Casey, Memoirs Coleop., vol. 4, p 36.
1916 *Cicindela macra* subsp. *topekana* Casey, Memoirs Coleop., vol. 7, p. 31.
1920 *Cicindela cuprascens* var. *macra* Leng, Cat. Coleop. Amer., p 42.
1930 *Cicindela cuprascens* var. *macra* Horn, Trans. Amer. Ent. Soc., vol. 56, p 86.

Length 11.5 to 13.5 mm. Dorsum dull greenish brown, faintly

bronzed; beneath metallic brassy green, trochanters rufous, palpi pale. Elytra densely but finely punctate; in the female emarginate externally at apex, forming an obtuse or rectangular subapical tooth, in the male feebly sinuate, tips prolonged slightly in both sexes. Markings complete, connected by marginal band, middle band often slender and tortuous; small spot at base of each elytron frequently connected to anterior portion of humeral lunule. Pubescence short, decumbent, moderately dense on front, occiput, and pronotum, very dense on cheek, thorax and sides of the venter.

Distinguished from *cuprascens* by the duller color, less strongly punctate elytra, the less distinct subapical tooth of female elytra, and by slight prolongation of elytral tip in both sexes.

This species is widely distributed in Iowa; more common in the east and southern parts. It frequents stream banks, pond and lake shores, showing a preference for silt covered bars exposed by receding water. During the hottest hours of the day, adults seek the protecting shade of sticks, stones and fissures in the soil. The dry summer of 1936 reduced the water level of many streams and exposed areas of wet, mud-covered sand on which *macra* were abundant in spite of the firm stand some distance from the water.

Occasionally this species is attracted to lights in towns situated along rivers. Adults appear early in July, live until about August 15. Evidently the life history does not differ from *cuprascens*.

County records: Bremer, VII, 1933 (Jaques); Clinton, VII, 1936 (Eckhoff); Johnson, VII, 1898 (Wickham); Linn, VII, 1929 (Fitz), VII, 1932 (Jay), VII, 1936 (Eckhoff); Louisa, VII, 1826 (H. M. Harris), VII, 1936 (Eckhoff); Monona, VII, 1932 (Russell), VII, 1932 (Moore), VII, 1936 (Eckhoff); Muscatine, VII, 1936 (Eckhoff); Pottawattamie, VI, 30, 1932 (Barker); Scott, VII, 1936 (Eckhoff); Van Buren, VII, 1936 (Eckhoff).

In addition to the above records Wickham (1911) records specimens from Black Hawk and Buchanan Counties.

CICINDELA LEPIDA Dej.

1831 *Cicindela lepida* Dejean, Species gen. Coleop. coll. Dejean, vol. 5, p 225.
1913 *Cicindela lepida* subsp. *insomnis* Casey, Memoirs Coleop., vol. 4, p 35.
1920 *Cicindela lepida* Leng, Cat. Coleop. Amer., p 42.
1930 *Cicindela lepida* Horn, Trans. Amer. Ent. Soc., vol. 56, p 86.

Length 10.5 to 13 mm. Head and thorax greenish bronze; elytra creamy white with a few green or brown bronzed lines or splotches, dispersedly punctate; beneath brown or greenish bronze, appendages pale. Head, thorax and abdomen densely clothed by short, decumbent hairs.

The species is very widely distributed but local and not abundant. It inhabits pure, usually dry sand such as might be found on the higher river banks or in the blowouts of sandy regions. Apparently nocturnal, it is collected near artificial lights at night where it remains in the semi-darkness near an area of illumination.

The adults appear early in July, feed only while cool temperatures prevail and spend the greater part of the hot daylight hours hidden under the surface of the sand.

County records: Story, (Ames, Iowa, Osborn).

Wickham (1911) records this species from Johnson and Pottawattamie Counties.

CICINDELA FORMOSA Say

1817 *Cicindela formosa* Say, Jour. Acad. Nat. Sci. Phila., vol. 1, p 19.
1913 *Cicindela formosa* subsp. *luxuriosa* Casey, Memoirs Coleop., vol. 4, p 24.
1920 *Cicindela formosa* Leng, Cat. Coleop. Amer., p 40.
1930 *Cicindela formosa* Horn, Trans. Amer. Ent. Soc., vol. 56, p 79.

Length 15 to 17 mm. Color above, cupreous red, often with a brassy luster, especially on the thorax; beneath, metallic blue with greenish reflections. Elytra feebly or not at all punctate, often granulate at the base; markings complete, connected at the margin, middle band transverse or only slightly deflexed. Front and ventral parts densely pubescent; prothorax bare dorsally, pilose at the lateral margins.

With no confirmatory evidence *formosa* has been included as a probable resident of extreme northwestern Iowa where climatic conditions and topography practically duplicate their Nebraska habitat. *Formosa* and *lengi* frequently occur together.

Seekers of this gaudy and wary beetle must scour the sand dunes and blowouts of a dry to semi-arid country during May and early June where it may be found hunting near, or concealed in, the shade of sparse vegetation surrounding the areas of bare drift sand. Easily alarmed, this species will run into an open area and readily exercise its strong powers of flight.

The life history given for var. *generosa* applies to this species.

There are no positive Iowa records to date.

CICINDELA PURPUREA Oliv.

1790 *Cicindela purprea* Olivier, Entomologie, vol. 2, p 14.
1801 *Cicindela marginalis* Fabricius, Systema Eleutheratorum, vol. 1, p 240.
1857 *Cicindela spreta* LeConte, Trans. Amer. Philos. Soc., ser. 2, vol. 11, p 37.
1920 *Cicindela ramosa* Gistel (in litt.) Leng, Cat. Coleop. Amer., p 40.
1920 *Cicindela superla* Dahl (in litt.) Leng, Cat. Coleop. Amer., p 40.
1920 *Cicindela purpurea* Leng, Cat. Coleop. Amer., p. 40.
1930 *Cicindela purpurea* Horn, Trans. Amer. Ent. Soc., vol. 56, p 79.
1932 *Cicindela purpurea* Nicolay & Weiss, Jour. N. Y. Ent. Soc., vol. 40, p 345.

Length 13 to 15 mm. Color above wine red to cupreous red, sutures and margins metallic blue or green. Lateral plates of thorax metallic cupreous bronze with green sutures, legs bronze, abdomen and ventral portions of head and thorax bluish green. Elytra slightly granulate; shallowly punctate; markings reduced to a short oblique middle band, apical and small anteapical spot. Prothorax distinctly narrowed at base; pubescence erect, moderately dense on front, thorax and legs.

Typical *purpurea* has been included in the Iowa list although no specimens have been observed. Wickham (1911) recorded *purpurea;* however, he may have collected the green variety, now called *auduboni,* and classified it as *purpurea.*

Auduboni occurs in the state and it is possible that true *purpurea* may be found. Shelford (1917) plate 27, extends the range of *purpurea* westward into eastern Iowa.

Life history and habits related for *auduboni* apply to this species.

County records: King (1914) records this species from Henry County; Wickham (1911) records this species from Howard, Johnson and Story Counties.

CICINDELA LONGILABRIS Say

1824 *Cicindela longilabris* Say, Keating's Exp. to Source of St. Peters River, App. vol. 2, p 16.
1837 *Cicindela albilabris* Kirby, The Insects in Richardson's Fauna Boreali-Americana, vol. 4, p 12.
1920 *Cicindela longilabris* Leng, Cat. Coleop. Amer., p 41.
1930 *Cicindela longilabris* Horn, Trans. Amer. Ent. Soc., vol. 56, p 82.

Length 13 to 16 mm. Color above dark brown or black; beneath dark metallic blue, often with greenish reflections. Elytra roughly granulate punctate; marking inconspicuous, composed of a narrow, occasionally broken, sinuate middle band, humeral, posthumeral and anteapical spots. Pubescence above consists of a few setae at the sides of the thorax, on the antennal scape and beside the eyes; beneath, distant erect hairs appear on the thoracic pleura, abdomen and appendages. Characterized by the elongate labrum and peculiar depression or excavation above the front between the eyes.

This species has not been recorded in Iowa; however, there are records from Wisconsin and Illinois, Leng (1920); Minnesota, Horn (1928); and Nebraska, Horn (1930).

Wickham (1898) found the species at Bayfiield, Wisconsin, during June and July frequenting hard, bare spots in woodland roads or paths. Dr. J. L. LeConte says that it frequents paths in grassy and bushy places, taking refuge in herbage if disturbed.

CICINDELA FULGIDA Say

1823 *Cicindela fulgida* Say, Jour. Acad. Nat. Sci. Phila., vol. 3, p 141.
1920 *Cicindela fulgida* Leng, Cat. Coleop. Amer., p 41.
1930 *Cicindela fulgida* Horn, Trans. Amer. Ent. Soc., vol. 56, p 82.

Length 11 to 12.5 mm. Color above polished brilliant to blackish red; beneath, light metallic green. Elytra densely and coarsely punctate, punctures without metallic colors. Markings of elytron broad but not connected, composed of an obliquely elongate humeral lunule, a sinuate middle band without a marginal line and a complete apical lunule. Pubescence of thorax, palpi, and legs strong, bristle like; of front dense, virtually cropped. Labrum inconsistently dentate, variable from distinctly tridentate to feebly one toothed.

In view of the data given below, this species has been listed as possibly present in Iowa, however, the wegiht of evidence is not highly convincing.

Horn (1928) lists *fulgida* occurring in Minnesota from an unrecorded locality. Horn (1930) gave the distribution "between New Mexico, Iowa, Manitoba, Montana and Colorado." The Nebraska collection contains many specimens taken at the edge of the salt flats near Lincoln.

CICINDELA PATRUELA Dej.

1825 *Cicindela patruela* Dejean, Species gen. Coleop. coll. Dejean, vol. 1, p 62.
1920 *Cicindela montana* Hentz (in litt.) Leng, Cat. Coleop. Amer., p 41.
1920 *Cicindela patruela* Leng, Cat. Coleop. Amer., p 41.
1930 *Cicindela sexguttata* var. *patruela* Horn, Trans. Amer. Ent. Soc., vol. 56, p 83.

Length 13 to 15 mm. Color above dull green, beneath metallic bluish green. Elytra coarsely granulate, obscurely punctate; marked by a slightly deflexed middle band, humeral, posthumeral, anteapical and apical spots. Front glabrous; prothorax moderately pubescent beneath; metasternum and met-episterna densely pilose; abdomen with few hairs. Form more robust than that of *sexguttata* with which it is sometimes confused.

No Iowa records of *patruela* have been obtained; however, it has been recorded from Minnesota, Horn (1928), who also gives the distribution "between Georgia, Vermont, Minnesota and Colorado" in his 1930 list. Specimens collected by the author at Ansley, Nebraska, April, 1934, verify a greater westward range than previously suspected. Here the adults appeared during April on the warm side of clay hills which supported scattered clumps of blue stem grass and patches of plum brush. Eastern specimens are found on grassy hillsides in or near wooded areas; on rough, stony, semi-barren soil; or on bare spots in woods where they behave somewhat like *sexguttata*.

CICINDELA RUFIVENTRIS Dej.

1825 *Cicindela rufiventris* Dejean, Species gen. Coleop. coll. Dejean. vol. 1, p 102.
1913 *Cicindela rufiventris* subsp. *collusor* Casey, Memoirs Coleop., vol. 4, p 39.
1920 *Cicindela rufiventris* Leng, Cat. Coleop. Amer., p 42.
1930 *Cicindela rufiventris* Horn, Trans. Amer. Ent. Soc., vol. 56, p 84.

Length 9 to 11 mm. Color above dark brown, with a sooty appearance caused by the large, shallow, greenish blue elytral punctures; head and prothorax bronze above, metallic blue beneath; abdomen reddish or orange. White markings of elytra inconspicuous, composed of humeral, marginal, anteapical and apical dots, in addition to the oblique, often broken middle band. Front glabrous, pubescence beneath sparse, largely decumbent.

Apparently a localized species sporadically collected in eastern United States it has been taken in Nebraska thus extending the range westward across Iowa although there are no Iowa records.

The species occurs on "knobs" and upland hills usually on clay or sandy clay soils which supports stunted vegetation. It was observed and collected by the author near Ansley, Nebraska, (April, 1934) on the sides of steep, sandy clay hills where is scurried about the open spaces between grass clumps.

LITERATURE CITED

(References marked with an asterisk have not been verified in the original.)

BALDUF, W. V.
1925. The Feeding of a Common Tiger Beetle, (Colepotera, Cicindelae). Ent. News, 36: 275-276.

CARR, F. S.
1920. Albertan Coleoptera. Can. Ent., 52: 218-220.

CASEY, THOMAS L.
1897. Coleopterological Notices VII. Ann. N. Y. Acad. of Sci., 9: 285-684.
1909. Studies in the Caraboidea and Lamellicornia. Can. Ent., 41: 253-284.
1913. Studies of the Cicindelidae and Carabidae of America. Memoirs on the Coleoptera, New Era Printing Co., Lancaster, Pa., 4: 1-192 p.
1914. Studies in *Omus* and *Cicindela*. Memoirs on the Coleoptera, New Era Printing Co., Lancaster, Pa., 5: 1-24.
1914. Miscellaneous Notes on New Species. Memoirs on the Coleptera, New Era Printing Co., Lancaster, Pa., 5: 355-378.
1916. Further Studies in the Cicindelae. Memoirs on the Coleoptera, New Era Printing Co., Lancaster, Pa., 7: 1-34.
1924. Additions to the Known Coleoptera of North America. Memoirs on the Coleoptera, New Era Printing Co., Lancaster, Pa., 11. 336 pp. (Cicindelidae p 1-16).

CRIDDLE, NORMAN.
1907. Habits of Some Manitoba "Tiger Beetles" *(Cicindela)*. Can. Ent., 39: 105-114.
1910. Habits of Some Manitoba Tiger Beetles (Cicindelidae). Can. Ent., 42: 9-15.
1925. A New *Cicindela* from the adjacent territory of Montana and Alberta. Can Ent., 57: 127-128.

DAVIS, C. ABBOTT.
1903. The Cicindelidae of Rhode Island. Ent. News, 14: 270-273.

DAWSON, R. W.
1928. Biology of the Tiger Beetles with a Key to the Species of *Cicindela* found in Minnesota. Minn. Agr. Exp. Sta. Tech. Bull. 56: 1-13.

DEJEAN, COMTE F. M. A. PIERRE.
*1825. Spécies général des Coléoptères de la collection de M. le comte Dejean. Paris, Mequignon Marvis, 1, xxx, 463 pp.
*1826. Spécies général des Coléoptères de la collection de M. le comte Dejean. Paris, Mequignon Marvis, 2, viii, 561 pp.
*1831. Spécies général des Coléoptères de la collection de M. le comte Dejean. Paris, Mequignon Marvis, 5, vii, 520 pp.

EMMONS, EBENEZER.
1854. Natural History of the Agriculture of New York. (Insects.) Albany, 5, 272 pp., 47 pl. (Cicindelidae pp. 32-38).

FABRICIUS, JOH. CHRIST.
1775. Systema Entomologica. Flensburgi and Lipsiae, Korte, 832 pp. (Cicindelidae, pp. 224-226).
1798. Supplementum Entomologiae Systematicae. Hafniae, Proft and Storch, 572 pp. (*Cicindela* pp. 60-63).
1801. Systema Eleutheratorum. Kiliae, Bibliopal. Acad., 1, (*Cicindela*, pp. 231-244).

FALL, H. C.
1901. A New *Cicindela*, with Notes on Allied Species. Ent. News, 12: 307-310.

FROST, C. A.
1920. Notes on the Coleoptera with Descriptions on New Species. Can. Ent., **52**: 220-232.

GISTEL, JOHANNES.
*1837. Systema Insectorum, secundum classes, ordines, genera, species, cum characteribus, synonymis annotationibus, locis et inconibus. Copeoptera. Munich, Fleischman, **1**: (1)16-64.

GOLDSMITH, WM. M.
1916. Field Notes on the Distribution of Life Habits of the Tiger Beetles (Cicindelidae) of Indiana. Proc. Indiana Acad. Sci. 1916, pp. 447-455.

GOULD, AUGUSTUS A.
1834. Art. IV, on the Cicindelidae of Massachusetts. Boston Jour. Nat. Hist., **1**: 41-54.

HALDEMAN SAMUEL S.
1853. Descriptions of Some New Species of Insects with Observations on Described Species. Proc. Acad Nat. Sci. Phila., **6**: 361-365.

HAMILTON, CLYDE C.
1925. Studies on the Morphology, Taxonomy and Ecology of the Larvae of Holarctic Tiger-Beetles. Proc. U. S. National Museum, Art. 17, **65**: 1-87.

HARRIS, EDWARD DOUBLEDAY.
1911. List of North American Cicindelidae in the Harris Collection. Yonkers, N. Y., 68 pp.

HENTZ, NICHOLAS M.
1830. Description of Eleven New Species of North American Insects (Coleopt.). Trans. Amer. Philos. Soc. (2) **3**: 253-258. (Reprinted in Bull. Brooklyn Ent. Soc., **6**: 109-112.

HERBST, JOHANN F. W.
*1806. Natursystem aller bekennten in- und auslandischen Insecten; nach dem System des Ritters Carl von Linne bearbeitet (Von C. G. Jablonsky), fortgesetzt von J. F. W. Herbst. Berlin, Pauli, **10**.

HOPE, F. W.
*1838. The Coleopterests Manual, containing the Lamellicorn Beetles of Linnaeus and Fabricius. London, Bohn, **2**: 168 pp.

HORN, WALTHER.
1908. Synonymische Bemerkungen uber Cicindelen-Arten. Deutsche Ent. Zeit., p. 738.

1915. Cicindelinae. Genera Insectorum (Coleoptera), **13**: 209-486, 23 pl. (Fasc. 82c).

1928. Notes and Records on the Tiger Beetles of Minnesota. Minn. Agr. Exp. Sta. Tec. Bull. 56: 9-13.

1930. Notes on the Race *Omus californicus* and a List of the Cicindelidae of America North of Mexico. (Coleoptera) Trans. Amer. Ent. Soc., **56**: 73-86, 1 pl.

KING, INEZ NAOMI.
1914. The Coleoptera of Henry County, Iowa. Proc. Iowa Acad. Sci., **12**: 317-340.

KIRBY, WILLIAM.
1837. Fauna Boreali-Americana by John Richardson. London, Longman etc., 4: (4)8-12.

KLUG, JOHANN C. F.
*1834. Jahrbücher der Insectenkunde mit besonderer Rücksicht auf die sammlung in Königl. Museum in Berlin herausgegeben. Berlin, **1**: 296.

KNAUS, WARREN.
1922. Two New Forms of *Cicindela* with Remarks on Other Forms. Jour. N. Y. Ent. Soc., **30**: 194-197.

1924. A Color Form of *Cicindela repanda-unijuncta*. Jour. N. Y. Ent. Soc., 32: 126.

1927. A New Name for *Cicindela violaceae* Fab. Jour. Kan. Ent. Soc., 2: 23-24.

1930. Notes on Kansas Coleoptera. Jour. Kan. Ent. Soc., 3: 79-80.

KOLLAR, VINCENZ.

*1836. Species Insectorum Coleopterorum novae. Annal. d. Wein. Mus., 1: 327-336.

LECONTE, JOHN L.

1845. Some New and Interesting Insects Inhabiting the U. S. Boston Jour. Nat. Hist., 5: 203-209.

*1848. A descriptive Catalogue of the Geodephagous Coleoptera inhabiting the United States east of the Rocky Mountains. Ann. Lyc. Nat. Hist. N. Y., 4: 173-474.

1852. Remarks on Some Coleopterous Insects Collected by S. W. Woodhouse, M. D., in Missouri Territory and New Mexico. Proc. Acad. Nat. Sci. Phila., 6: 65-68.

*1857. Revision of the Cicindelidae of the U. S. Trans. Amer. Philos. Soc., (2) 11: 27-62.

1886. Additions to the Coleopterous Fauna of the United States. No. 1, Proc. Acad. Nat. Sci. Phila., 18: 361-394.

LENG, CHAS. W.

1902. Revision of the Cicindelidae of Boreal America. Trans. Amer. Ent. Soc., 28: 93-186, 4 pl.

1910. Notes on Coleoptera Collected in Northern Georgia. Jour. N. Y. Ent. Soc., 18: 71-82.

1912. The Geographical Distribution of Cicindelidae in Eastern North America. Ent. News., 20: 1-17.

1918. A New Race of *Cicindela* with Notes on Other Races and Species. Jour. of N. Y. Ent. Soc., 26: 138-141.

1920. Catalogue of the Coleoptera of America North of Mexico. Mt. Vernon, N. Y., John D. Sherman, Jr., 470 pp.

1933. Second and Third Supplements to Cat. of Coleop. of Amer. North of Mexico (1925 to 1932 inc.). Mt. Vernon, N. Y., John D. Sherman, Jr., 112 pp.

LINNAEUS, CARL.

*1758. Systema Naturae Ed. 10. Holmiae, 1, Coleoptera, pp. 345-433.

*1766. Systema Naturae Ed. 12. Holmiae, 1, 532 pp.

1788. Systema Naturae Ed. 13. Lipsiae, 1, (4) (Ed. by Gmelin).

LJUNGH, SVEN I.

*1799. Nya Insecta utur egen Sammling beskrifne. *(Scarabaeus Mormon, Chrysomela bivittata, cicindela varians, Papilio Brigitta.)* Vetensk. Acad. Nya Handl., 20: 145-149.

MACNAMARA, CHARLES.

1922. Tiger Beetle Larvae. Can. Ent. 54: 241-246.

MELSHEIMER, F. VALENTIN.

*1806. Catalog of the Insects of Pennsylvania. Hanover, York County, pp. 1-66.

MICHELSON, ALBERT ABRAHAM.

*1911. On Metallic Colouring in Birds and Insects. Philos. Mag. London, 21: 554-567.

MONTGOMERY, B. ELWOOD.

1930. Records of Indiana Coleoptera. I. Cicindelidae. Proc. Ind. Acad. Sci., 40: 357-359.

NICOLAY, ALAN S., and HARRY B. WEISS.

1932. Synopsis of the Cicindelidae Part I; General Introduction, Bibliography and Purpurea Group. Jour. N. Y. Ent. Soc., 40: 341-355.

OLIVIER, ANTOINE G.

*1790. Entomologie, ou histoire naturelle des insects, avec leurs caracteres génériques et spécifiques, leur description, leur synonymie et leur figure enluminée. Coléoptères. Paris, Bandonin, 2: 485 pp., 63 pl.

RIDDLE, OSCAR.

1909. Our Knowledge of Melanin Color Formation and Its Bearing on the Mendelian Description of Heredity. Biol. Bull. Marine Biol. Lab., 16: 316-351.

SAY, THOMAS.

*1817. Description of Several New Species of North American Insects. (5 *Cicindela*). Jour. Acad. Nat. Sci. Phila., 1: 19-23. (Reprinted in his complete writings, 2: 1-4, 1883.)

*1818. Monograph of the North American Insects of the genus *Cicindela*. Trans. Amer. Philos. Soc. new ser., 1: 401-426.

*1823. Descriptions of Coleopterous Insects collected in the Expedition to the Rocky Mountains. Jour. Acad, Nat. Sci. Phila., 3: 139-216.

1824. Keatings narrative of an expedition to the source of St. Peters River, Lake Winnepeck, Lake of the Woods, under the command of Major Long 1823. Appendix after 2: (class Insecta) 16-194.

SCHAUPP, FRANS G.

1883. Synoptic Tables of the Coleoptera (Cicindelidae). Bull. Brooklyn Ent. Soc., 6: 73-108.

SHELFORD, VICTOR E.

1907. Preliminary Note on the Distribution of the Tiger Beetles (*Cicindela*) and Its Relation to Plant Succession. Biol. Bull. Marine Biol. Lab., 14: 9-14.

1908. Life-Histories and Larval Habits of the Tiger Beetles (Cicindelidae). Jour. Linn. Soc. Zoology. London, 30: 157-184, 4 pl.

1911. Physiological Animal Geography. Jour. of Morphology, 22: 551-618, 4 pl.

1917. Color and Color-Pattern Mechanism of Tiger Beetles. Illinois Biol. Monog., 3: 395-528.

THOMPSON, WM. C.

1915. *Cicindela unipunstata* at Seaville, N. J., (Coleoptera). Ent. News, 26: 425-426.

VARAS, ARANGUA.

1928. IV Contribucion al Estudio de los Cicindelidae Notas sobre las variedades de Algunas especies de Cicindela del grupo—formosa purpurea oregona-. Rev. Chil. Hist. Nat., 32: 231-251.

WEISS, HARRY B.

1932. See Nicolay and Weiss.

WICKHAM, H. F.

1898. The Habits of American Cicindelidae. Proc. Davenport Acad. Sci., 7: 206-228.

1906. The Races of *Cicindela tranquebarica* Hbst. Ent. News, 17: 43-48.

1911. A List of the Coleptera of Iowa. Iowa Univ. Lab. Nat. Hist. Bull., 6! (2) 1-40.

THE DISTRIBUTION OF SYMMETRIC QUADRATIC FORMS IN NORMAL AND INDEPENDENT VARIABLES[1]

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Let $x_1, x_2, \ldots, x_N$ be a set of N normally and independently distributed variables. The joint distribution function of the x_i is:

$$\text{(1)} \quad f(x_1, x_2, \ldots x_N)\, dx_1\, dx_2 \ldots dx_N = (2\pi)^{-\frac{1}{2}N} e^{-\frac{1}{2}(x^2_1 + x^2_2 + \ldots + x^2_N)}\, dx_1\, dx_2 \ldots dx_N$$

We define a symmetric quadratic form in the variables x_i, i. e.,

$$\text{(2)} \quad Q = a(x^2_1 + x^2_2 + \ldots + x^2_N) + 2b(x_1x_2 + x_1x_3 + \ldots + x_{N-1}x_N),$$

where the coefficient of all the squares, a, and the coefficient of all the products, b, are real numbers.[3] We want to establish the distribution of the quadratic form Q by means of the method of characteristic functions.[4]

The characteristic function of y, say g(y), is definied as:

$$\text{(3)} \quad g(y) = \int_{-\infty}^{+\infty} \ldots \int_{-\infty}^{+\infty} e^{iyQ} f\, dx_1 \ldots dx_N$$

$$= \int_{-\infty}^{+\infty} \ldots \int_{-\infty}^{+\infty} (2\pi)^{-\frac{1}{2}N} e^{iya(x^2_1 + \ldots + x^2_N) + 2iyb(x_1x_2 + \ldots + x_{N-1}x_N)}\, e^{-\frac{1}{2}(x^2_1 + \ldots + x_N)^2} dx_1 \ldots dx_N.$$

The exponent of the integrand appears as:

$$\text{(4)} \quad -\tfrac{1}{2}[(1 - 2iay)(x^2_1 + \ldots + x^2_N) - 4iby(x_1x_2 + \ldots + x_{N-1}x_N)].$$

The expression in brackets can be transformed by an orthogonal transformation with the following determinant:

$$\text{(5)} \quad D = \begin{vmatrix} (1-2iay), & -2iby, & \ldots, & -2iby \\ -2iby, & (1-2iay), & \ldots, & -2iby \\ \ldots & \ldots & \ldots & \ldots \\ -2iby, & -2iby, & \ldots, & (1-2iay) \end{vmatrix} = \{1-2iy[a+(N-1)b]\} \cdot [1-2iy(a-b)]^{N-1}.$$

The characteristic function is finally, by integration:

$$\text{(6)} \quad g(y) = D^{-\frac{1}{2}} = \{1 - 2iy[a + (N-1)b]\}^{-\frac{1}{2}}[1 - 2iy(a - b)]^{-\frac{1}{2}(N-1)}.$$

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[3] M. S. Bartlett and J. Wishart: The Distribution of Second Order Moment Statistics in a Normal System, Proceedings Cambridge Philosophical Society, vol. 28, 1931, pp. 455 ff.

[4] S. S. Wilks: Statistical Inference. Princeton, 1937, pp. 34 ff.

The distribution of Q, say D(Q) dQ, is established in the following manner:

$$(7)\qquad D(Q)=\frac{1}{2\pi}\int_{-\infty}^{+\infty} e^{-iQy}g(y)\,dy$$

$$=\frac{1}{2\pi}\int_{-\infty}^{+\infty} e^{-iQy}\{1-2iy[a+(N-1)b]\}^{-\frac{1}{2}}[1-2iy(a-b)]^{-\frac{1}{2}(N-1)}dy.$$

The integrand in (7) can be transformed in the following way:

$$(8)\qquad e^{-iQy}\{1-2iy[a+(N-1)b]\}^{-\frac{1}{2}}[1-2iy(a-b)]^{-\frac{1}{2}(N-1)}$$

$$=e^{-iQy}[a+(N-1)b]^{-\frac{1}{2}}(a-b)^{-\frac{1}{2}(N-1)}\left(\frac{1}{a+(N-1)b}-2iy\right)^{-\frac{1}{2}}\cdot\left(\frac{1}{a-b}-2iy\right)^{-\frac{1}{2}(N-1)}$$

$$=e^{-iQy}[a+(N-1)b]^{-\frac{1}{2}}(a-b)^{-\frac{1}{2}(N-1)}\left(\frac{1}{a+(N-1)b}-2iy\right)^{-\frac{1}{2}}\cdot\left(\frac{Nb}{(a-b)[a+(N-1)b]}+\frac{1}{a+(N-1)b}-2iy\right)^{-\frac{1}{2}(N-1)}$$

$$=e^{-iQy}[a+(N-1)b]^{-\frac{1}{2}}(a-b)^{-\frac{1}{2}(N-1)}\left(\frac{1}{a+(N-1)b}-2iy\right)^{-\frac{1}{2}N}\cdot\left\{1+\left(\frac{Nb}{(a-b)[a+(N-1)b]}\right)\left(\frac{1}{a+(N-1)b}-2iy\right)^{-1}\right\}^{-\frac{1}{2}(N-1)}$$

The last term in braces can be developed in terms of

$\left(\frac{1}{a+(N-1)b}-2iy\right)^{-1}$ and (8) becomes:

$$(9)\qquad e^{-iQy}[a+(N-1)b]^{-\frac{1}{2}}(a-b)^{-\frac{1}{2}(N-1)}\left\{\left(\frac{1}{a+(N-1)b}-2iy\right)^{-\frac{1}{2}N}\right.$$

$$-\frac{(\frac{1}{2}N-\frac{1}{2})}{1!}\left(\frac{Nb}{(a-b)[a+(N-1)b]}\right)\left(\frac{1}{a+(N-1)b}-2iy\right)^{-\frac{1}{2}N-1}$$

$$+\frac{(\frac{1}{2}N-\frac{1}{2})(\frac{1}{2}N+\frac{1}{2})}{2!}\left(\frac{Nb}{(a-b)[a+(N-1)b]}\right)^{2}$$

$$\left.\cdot\left(\frac{1}{a+(N-1)b}-2iy\right)^{-\frac{1}{2}N-2}-\ldots\ldots\ldots\right\}$$

This series can be integrated term by term if we remember that by the theorm of residues:[5]

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[5] See also G. A. Campbell and R. M. Foster: Fourier Integrals for Practical Applications. New York, p. 44, formula 431. 1931.

$$(10)\quad \int_{-\infty}^{+\infty} e^{-iQy}\left(\frac{1}{a+(N-1)b}-2iy\right)^{-m} dy$$
$$=\frac{-\pi}{(m-1)!}\left(\frac{Q}{2}\right)^{m-1} e^{-Q/2[a+(N-1)b]}.$$

Hence the distribution of Q finally becomes:

$$(11)\quad D(Q)=\frac{-e^{-Q/2[a+(N-1)b]}}{2\pi\sqrt{[a+(N-1)b](a-b)^{N-1}}}$$
$$\cdot\left\{\frac{(\tfrac{1}{2}Q)^{\frac{1}{2}N-1}}{(\tfrac{1}{2}N-1)!}-\frac{(\tfrac{1}{2}N-\tfrac{1}{2})(\tfrac{1}{2}Q)^{\frac{1}{2}N}}{1!(\tfrac{1}{2}N)!}\left(\frac{Nb}{(a-b)[a+(N-1)b]}\right)\right.$$
$$\left.+\frac{(\tfrac{1}{2}N-\tfrac{1}{2})(\tfrac{1}{2}N+\tfrac{1}{2})(\tfrac{1}{2}Q)^{\frac{1}{2}N+1}}{2!(\tfrac{1}{2}N+1)!}\left(\frac{Nb}{(a-b)[a+(N-1)b]}\right)^2-\ldots\right\}$$
$$=\frac{-e^{-Q/2[a+(N-1)b]}(\tfrac{1}{2}Q)^{\frac{1}{2}N-1}}{2\pi(\tfrac{1}{2}N-1)!\sqrt{[a+(N-1)b](a-b)^{N-1}}}$$
$$\cdot{}_1F_1\left(\frac{N}{2}-\frac{1}{2};\frac{N}{2};\frac{-NbQ}{2(a-b)[a+(N-1)b]}\right)$$

where ${}_1F_1$ denotes the generalized hypergeometric function.[6]

Define $F_\alpha(z)=\dfrac{(z/2)^{\frac{1}{2}\alpha-1}e^{-\frac{1}{2}z}}{2(\tfrac{1}{2}\alpha-1)!}$ the distribution of $z=\chi^2$ for α degrees of freedom.[7] Then the distribution of Q can be expressed in terms of these distribution.[8]

$$(12)\quad -\sqrt{\frac{[a+(N-1)b]^{N-8}}{(a-b)(Nb)^{N-2}}}\left\{F_N\left(\frac{Q}{(a-b)[a+(N-1)b]}\right)\right.$$
$$-\frac{(\tfrac{1}{2}N-\tfrac{1}{2})(Nb)}{1!}F_{N+2}\left(\frac{Q}{(a-b)[a+(N-1)b]}\right)$$
$$\left.+\frac{(\tfrac{1}{2}N-\tfrac{1}{2})(\tfrac{1}{2}N+\tfrac{1}{2})(Nb)^2}{2!}F_{N+4}\left(\frac{Q}{(a-b)[a+(N-1)b]}\right)-\ldots\right\}$$

This form is probably the most convenient one for calculations, since the χ^2 distribution has been frequently tabulated.[9]

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[6] G. N. Watson: A Treatise on the Theory of Bessel Functions. Cambridge, p. 100. 1922.

[7] S. S. Wilks: *op. cit.*, p. 32.

[8] This formula is due to Mr. S. S. Wilks.

[9] See e. g. R. A. Fisher: Statistical Methods for Research Workers. 4th ed., London, pp. 104 f. 1932.

THE EFFECT OF THE INGESTION OF IRRADIATED YEAST, MOLDS, AND ERGOSTEROL ON THE ANTIRACHITIC POTENCY OF EWE'S MILK[1]

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It is known that the ingestion of antirachitic supplemental feeds by the cow greatly increases the vitamin D potency of her milk, but no data are available showing the effects of antirachitic feeds on the vitamin D potency of the milk of the ewe. The experiment reported herein, and a similar one conducted simultaneously with cows (4), was undertaken to determine the effect of supplemental feeding of irradiated yeast[3], molds and ergosterol to ewes and also the effect of exposure of ewes to sunshine and ultra-violet light on the vitamin D activity of their milk. Criteria for the effect of the antirachitic feeds and irradiation were the rate of increase and maximum increase in the vitamin D content of the butter fat during the supplemental period, and the rate of decrease during the post-supplemental period.

PRELIMINARY EXPERIMENT

Three lactating ewes, A, B, and C selected from the main flock, were used during the preliminary experiment. Throughout the experimental period they were kept inside and fed a basal ration of alfalfa hay, corn, and oats.

Ewe A was given a supplemental feeding of 7,895 Steenbock rat units (3) of vitamin D as irradiated yeast daily for 8 days. Ewe B received 21,429 rat units of vitamin D as irradiated molds daily for 28 days, whereas ewe C was exposed to the ultra-violet rays from a Cooper-Hewitt mercury vapor lamp for 30 minutes daily throughout the 28 days.

It was found by biological assays that 9.5 grams of butter fat, prepared from a composite sample of milk from ewes A, B, and C collected previous to the feeding of irradiated yeast and molds or direct irradiation, were equivalent to one Steenbock rat unit of vitamin D. An assay of the butter fat produced by ewe A on the seventh and eighth days of the supplemental period showed that 1.2 grams contained more than a rat unit of vitamin D. This was more than a seven-fold increase in the vitamin D potency over that of the pre-supplemental butter fat. A sample of butter fat prepared from the milk of ewe B collected on the twenty-third, twenty-fourth, twenty-fifth, and twenty-sixth days of the supplemental period showed that 0.25 gram was equivalent to a rat unit of vitamin D. This was a thirty-seven-fold increase in the antirachitic activity of the butter

[1] Journal Paper Number J-634 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 96.

[2] Abstract of author's Doctoral Thesis No. 289. Library, Iowa State College.

[3] The dried molds and irradiated ergosterol, and the yeast used in these experiments were contributed by Acetol Products, Inc., New Brunswick, N. J., and the Fleischmann Laboratories, New York, N. Y., respectively.

fat produced by feeding irradiated yeast. Eight grams of butter fat prepared from the milk produced by ewe C at the end of the third and fourth weeks of the irradiation period when fed to rachitic rats did not cause any calcification in the rachitic metaphyses of the radii and ulnae. An assay of a composite sample of butter fat prepared from milk produced by the main flock, which was on summer pasture, indicated that 9.5 grams contained more than one rat unit while 8.5 grams contained less than one rat unit of vitamin D.

EXPERIMENTAL

The results obtained in the preliminary test were used as a basis in outlining the main experiment. Since experimental work had shown that the ingestion of irradiated ergosterol by cows was a means by which the vitamin D content of their milk could be increased the experiment was enlarged to include a group of ewes fed this antirachitic supplement.

Nineteen grade Hampshire ewes, from 3 to 6 years of age, weighing from 150 to 210 pounds, were used. Previous to lambing, all the ewes had access to winter pasture and in addition were fed a liberal allowance of alfalfa hay and a grain mixture consisting of corn and oats.

To facilitate the collection of milk from the lactating ewes the lambs were kept separate from their dams except for three hours immediately following the morning and evening milkings. Samples of butter fat were prepared from the milk obtained from each of the ewes fed irradiated yeast, molds, and ergosterol, and from samples of milk obtained at regular intervals throughout the test period from ewes fed no antirachitic supplement.

Rats which had been rendered rachitic were used for the biological assays of the various butter fat samples for vitamin D by the procedure outlined by Steenbock and co-workers (2). Butter fat from the milk of experimental ewes was fed to the rachitic rats by means of a 1 cc. pipette calibrated to deliver the fat at 35° to 40° C. The "line test" method developed by McCollum and associates (1) was used to determine the amount of vitamin D in the various samples of butter fat. Groups of six to ten rachitic rats were fed at the level of butter fat which most closely approximated a rat unit of vitamin D, and a similar number of rats were fed these butter fat samples simultaneously at levels 10 per cent above and 10 per cent below this amount.

As the 19 ewes lambed they were allotted in five groups A, B, C, D, and E, of three ewes each and two groups, F and G, of two ewes each. Groups A, B, C, D, and E were kept in a combination shed and dry lot, while group F was kept in a darkened room, and group G on pasture. Groups A, B, C, D, and E were maintained through three successive feeding periods; a pre-supplemental period of 6 to 12 days, a supplemental period of 35 days, and a post-supplemental period of 28 days. The basal ration consisted of alfalfa hay and two pounds per ewe per day of a grain mixture of ground corn 33.0, ground oats 66.5, and sodium chloride 0.5 parts by weight, respectively. Supplements to this grain mixture consisted of sufficient irradiated yeast or molds previously assayed, to supply the antirachitic factor in the desired amount. The irradiated ergosterol was added to enough corn oil to make up 2 per cent of the grain ration. Each ewe in groups A, B, and C was fed daily 2,500, 7,500, and 15,000 rat

units, respectively, of vitamin D as irradiated yeast, while each ewe in group D received 7,500 rat units of vitamin D as irradiated molds and group E 7,500 rat units of vitamin D as irradiated ergosterol.

It was shown by biological assays that 9.5 grams of the butter fat prepared from the milk produced by each group of ewes during the pre-supplemental period were equivalent to one rat unit of vitamin D. At the end of the fifth week of the supplemental period only 3.5 grams of butter fat from the ewes in group A, 1.4 grams from the ewes in group B, 0.65 gram from the ewes in group C, 1.32 grams from the ewes in group D, and 2.7 grams from the ewes in group E were required to equal one rat unit of vitamin D. The greatest increase in the vitamin D potency of the butter fat took place during the first eight days of the supplemental period and a further increase was at a decreasing rate. The irradiated yeast and molds when fed to lactating ewes at the 7,500 rat unit level were equally efficient as a means of increasing the antirachitic potency of the butter fat whereas irradiated ergosterol at the same level of feeding was about one-half as efficient.

The rate of decrease in the antirachitic potency of ewe's milk or butter fat during the first two weeks after supplemental feeding had been discontinued was very rapid. At the end of the four-week post-supplemental period the antirachitic activity of the butter fat approached but did not reach the low level of activity of the butter fat produced during the pre-supplemental period.

The ewes in group F were kept in a darkened room and on a dry feed for 81 days with no apparent change in the antirachitic activity of their butter fat. At the beginning and at the end of this period 9.5 grams of butter fat were required to equal one rat unit of vitamin D. At the end of this time their faces were irradiated for two hours daily throughout a 14-day period with a Cooper-Hewitt mercury vapor lamp. The butter fat collected on the thirteenth and fourteenth days of the irradiation period had a vitamin D potency such that 7.5 grams were equal to one rat unit. This indicated that exposing the faces of the ewes to direct ultra-violet rays was only slightly effective as a means of increasing the antirachitic activity of the milk. Exposing the ewes in group G to summer sunshine and green pasture did not change the antirachitic potency of their butter fat.

MILK PRODUCTION

Four ewes, A-2, A-3, B-2, and E-3, did not have lambs suckling them. Daily records were kept of the amount of milk produced by each. Samples of milk were taken for butter fat, calcium, and phosphorus determinations.

The average daily milk yield during the 35-day supplemental period was 894 grams for A-2, 1,050 grams for A-3, 2,301 grams for B-2, and 1,075 grams for E-3.

Butter fat determinations were made of the milk produced by the four ewes on three successive days during the pre-supplemental period, at the end of each week during the supplemental period, and at the end of the second and fourth weeks of the post-supplemental period. The percentage of butter fat in the milk ranged from 5.15 for the milk produced by A-3 at the end of the second week of the supplemental period to 9.18 for the milk produced by B-2 at the end of the fourth week of the post-

supplemental period. The day-to-day variations in the butter fat content of the milk collected during the pre-supplemental period were as great as the variations from week-to-week in the supplemental and post-supplemental periods.

Determinations of calcium and phosphorus content of the milk produced by each of the four ewes were made at the end of the pre-supplemental period, at the end of the second and fifth week of the supplemental period, and at the end of the fourth week of the post-supplemental period. The average calcium content of the milk produced by the four ewes for each of the above periods was 0.20, 0.20, 0.20, and 0.22 per cent, while the phosphorus content for these same four periods was 0.15, 0.16, 0.16, and 0.16 per cent. The percentage of calcium in the milk ranged from 0.18 for A-3 at the end of the pre-supplemental period to 0.23 for B-2 at the end of the fourth week of the post-supplemental period. The range in phosphorus content of the milk samples analyzed was from 0.12 per cent for B-3 for a sample collected in the pre-supplemental period to 0.19 per cent for A-3 at the end of the fourth week of the post-supplemental period. The variations in calcium and phosphorus content seemed to be due to the individuality of the ewes rather than to any dissimilarity in their care or ration.

CONCLUSIONS

Milk produced by nursing ewes contains small quantities of vitamin D. The exposure of the experimental ewes to summer feeding conditions did not influence the vitamin D content of their milk. The addition of irradiated yeast, irradiated molds or irradiated ergosterol to the ration of the lactating ewe definitely increased the antirachitic potency of the milk. Irradiated yeast and molds were used by lactating ewes with approximately equal efficiency to increase the antirachitic activity of the butter fat produced. Irradiated ergosterol was found to be approximately one-half as efficient for this purpose as irradiated yeast and molds when fed at equivalent levels. The antirachitic potency of the butter fat decreased rapidly after the vitamin D supplemental feeding was discontinued.

BIBLIOGRAPHY

1. McCollum, E. V., Nina Simmonds, P. G. Shipley, and E. A. Parks
1922. Studies on experimental rickets. XIV. A delicate biological test for calcium-depositing substances. Jour. Biol. Chem. **51**: 41-50.

2. Steenbock, H., and A. Black
1925. Fat soluble vitamins XXIII. The induction of growth-promoting and calcifying properties in fats and their unsaponifiable constituents by exposure to light. Jour. Biol. Chem. **64**: 263-298.

3. Steenbock, H., S. W. Kletzien, and J. G. Halpin
1932. The reaction of the chicken to irradiated ergosterol and irradiated yeast as contrasted with the natural vitamin D of fish liver oils. Jour. Biol. Chem. **97**: 249-264.

4. Thomas, B. H., and C. Y. Cannon
1934-35. Influence of diet on the antirachitic potency of cow's milk. An. Rept. Ia. Ag. Expt. Sta., p. 49.

NOTEWORTHY ADDITIONS TO THE COLLECTION OF MAMMALS FROM IOWA IN 1938[1]

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In 1937 a review of mammalian occurrences in Iowa was prepared (Scott, 1937) as an introduction to a proposed intensive investigation of intrastate distribution. This review included 63 species and subspecies of recent occurrence and 17 for which evidence of former or current existence might be shown with continued research. During the period, November 1, 1937, to November 1, 1938, approximately 400 specimens of mammals from Iowa were preserved in the Iowa State College Collection; among these, 15 species and subspecies have been identified as additions to the collection. This new material furnished evidence that changes in the State list were required.

All the material here represented, except the weasels, has been identified in the Section of Wildlife Surveys, United States Biological Survey. For this assistance acknowledgment is made to Dr. H. H. T. Jackson and A. H. Howell. The weasel specimens were identified by Dr. E. R. Hall, of the University of California.

So far as the writer knows, the three species and subspecies here listed have not been recorded previously from Iowa in a published report supported by specimens.

Pipistrellus subflavus subflavus (F. Cuvier)
GEORGIAN BAT

Allen (1871) listed the Georgian bat from Iowa on known distribution; Van Hyning and Pellett (1910) recorded it without comment. Miller (1897, p. 91) wrote of the distribution: "Austral zones and casually parts of Transition zone in Eastern United States, from the Atlantic Coast west to Iowa and eastern and southern Texas."

Specimens examined:

- Dubuque County, Dubuque; No. 116 a; Iowa State College Collection; No. 117a; now in U. S. Biological Survey Collection, No. 263853.
- Jackson County, Maquoketa, Maquoketa State Park; No. 90a; Iowa State College Collection.

Mustela rixosa subsp.
LEAST WEASEL

The remains of weasels from two pellets of the great horned owl *(Bubo virginianus virginianus)* have been referred to *Mustela rixosa*. One of the pellets was collected during the winter of 1933-34 from Section

[1] Journal Paper No. J-629 of the Iowa Agricultural Experiment Station. Project No. 498 In cooperation with the United States Biological Survey, the American Wildlife Institute, and the Iowa State Conservation Commission.

28, Highland Township, Wapello County; the other, a freshly regurgitated pellet, was collected at Emmetsburg, Palo Alto County, on May 16, 1934. These occurrences suggest an extensive intrastate distribution.

Mustela frenata primulina Jackson
MISSOURI WEASEL

The two specimens of the Missouri weasel that have been added to the Iowa State College Collection were taken from very near the zone of intergradation with *spadix*.

Specimens examined:

Boone County, Worth Township, Section 21; No. 119a; Iowa State College Collection.

Dubuque County, Mississippi River, 5 mi. SE of Dubuque; No. 140a (skull only); Iowa State College Collection.

REINTERPRETATION

Myotis subulatus subulatus (Say)
SAY BAT

Vespertilio subulatus Say, Long's Exped. Rocky Mts. vol. 2, p. 65.
Type Locality: Arkansas River near La Junta, Otero County, Colo.
Vespertilio subulatus Allen, 1871, p. 187; Osborn, 1890, p. 42.
Vesperitillio (sic) *subulatus* Goding, 1883, p. 330.
Myotis subulatus Van Hyning and Pellett, 1910, p. 215.

A preserved specimen of *subulatus* has been uncovered in the Iowa State College Museum to provide for inclusion of this bat in the State list. References to *subulatus* in former lists were interpreted as misidentifications of *Myotis keenii septentrionalis* and were included in the synonomy for this subspecies by Scott (1937). Reinterpretation is offered in the above synonomy. This synonomy is limited to the original description and to records from the lists of Allen (1871), Goding (1883), Osborn (1890), Van Hyning and Pellett (1910), Ruthven and Wood (1912), Stoner (1918), and Gabrielson (1921).

Osborn (1892) represented his list of Iowa mammals as having been supported by preserved specimens in the Iowa State College Museum. Osborn recorded *subulatus* in both his lists (1890 and 1892); however, like most of the specimens in support of Osborn's paper, the preserved specimen of *subulatus* could not be found. The specimen of *subulatus* recently uncovered in the museum is clearly tagged as having been taken at Ames on May 12, 1883, by A. M. Quist. As the date of collection of this specimen antedates Osborn's publication, it is more than probable that it is a specimen examined and preserved by him or under his direction. Van Hyning and Pellett (1910) are scarcely justified in listing *subulatus* as common throughout the State.

Miller and Allen (1928, p. 168) wrote of the distribution of this subspecies: "Arid plains and eastern Rocky Mountain region from Kansas and southeastern Colorado north to Montana."

Specimen examined:

Story County, Ames; No. 257a; now in U. S. Biological Survey Collection.

HYPOTHETICAL OCCURRENCE

Rangifer sp.
CARIBOU

A fragment of antler, probably from a brow tine of a caribou, was collected in a stream bed near Logan, Harrison County, by Charles Yocum. This brings up the question of the occurrence of this mammal in Iowa. The fragment was loaned to Iowa State College for examination and is preserved in the Yocum private collection.

REFERENCES CITED

ALLEN, J. A.
1871. Notes on the Mammals of Iowa. Boston Soc. Nat. Hist. Proc., **13**: 178-194.

GABRIELSON, IRA N.
1921. Notes on the Mammals Observed in Marshall County. Iowa Acad. Sci. Proc., **28**: 147-149.

GODING, W. F.
1883. Catalogue of the Mammals of Iowa. Iowa State Agr. Soc. (1882), pp. 329-331.

MILLER, GERRIT S., JR.
1897. Revision of the North American Bats of the Family Vespertilionidae. North American Fauna, No. 13.

MILLER, GERRIT S., JR., and GLOVER M. ALLEN
1928. The American Bats of the Genera Myotis and Pizonyx. U. S. Natl. Mus. Bul. 144, pp VIII, 218.

OSBORN, HERBERT
1890. Catalogue of the Mammals of Iowa. Iowa Acad. Sci. Proc., **1**: 41-44.

———
1892. A Partial Catalogue of the Animals of Iowa. Iowa Agr. College, pp. 1-39.

RUTHVEN, A. G., and N. A. WOOD
1912. Notes on a Collection of Mammals from Northwestern Iowa. Iowa Acad. Sci. Proc., **19**: 203-205.

SCOTT, THOS. G.
1937. Mammals of Iowa. Iowa State College Jour Sci., **12**: 43-97.

STONER, DAYTON
1918. The Rodents of Iowa. Iowa Geol. Survey Bul. **5**: 1-172.

VAN HYNING, T., and FRANK C. PELLETT
1910. An Annotated Catalogue of the Recent Mammals of Iowa. Iowa Acad. Sci Proc., **17**: 211-218.

BIOLOGICAL ASSAY OF FEEDING STUFFS IN A BASAL RATION FOR COCCIDIUM-GROWTH-PROMOTING SUBSTANCE[1]

III. DRIED FISH MEAL, ALFALFA MEAL, WHITE WHEAT FLOUR

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Received April 5, 1939

This is the third report of a series in the biological assay of feeding stuffs in basal ration for hypothetical coccidium-growth-promoting substance. The procedure for making the assays was that employed by Becker and Derbyshire (1937). W-values and F-values are to be defined as in the second report (1938) by the same authors.

ASSAY OF DRIED FISH MEAL

Whiting canned in tin and marketed for feeding poultry was purchased from the packer on the Atlantic Coast. According to information supplied by the distributor, the canned wet product contained 18.8 per cent protein. It was dried at 72° C. in an electric oven for 24 hours, ground to a powder in a coffee mill, and fed in the basal ration at the 10 per cent level. The dried product should have contained not less than 60 per cent protein.

TABLE 1. *Oöcyst counts and weight gains for rats on 4 per cent dried yeast and 10 per cent fish meal (dried "ATCO")*

		(1) Reference series		(2) Test series		Ratio (2):(1)	
Trial	Rat number	Weight gain (gm.)	Oöcysts (10^6)	Weight gain (gm.)	Oöcysts (10^6)	Weight gains	Oöcyst counts
1	1	Died	Died	26	22		
	2	36	107	20	32		
	3	43	76	22	27		
	4	39	39	19	19		
	5	40	99	17	10		
	6	33	35	25	23		
	7	43	53	10	19		
	8	44	61	24	18		
	Mean	39.7	67.14	20.4	21.25	0.51	0.32
2	9	22	93	14	58		
	10	27	146	19	17		
	11	17	175	8	30		
	12	24	141	15	46		
	13	25	104	10	21		
	Mean	23	131.8	13	34.4	0.57	0.26
W						0.534	
F							0.30

[1] Supported in part by grants from the American Academy of Arts and Sciences, and the Industrial Science Research Institute at Iowa State College.

Table 1 summarizes the results. The W-value of 0.534 indicates that growth was seriously impaired although the animals were on the rations only 16 days before the last weighing. Tests were not carried out to ascertain in what respects the fish was incomplete, since the immediate concern of the authors was with the effect on the parasite rather than the host. The F-value of 0.30 is presumed to indicate for the fish meal a paucity of the materials necessary for coccidium-growth. The F-value is very close to that of 0.311 previously reported (1937) for meat scraps, but the W-value is almost 5 times as high as for the meat scraps.

ASSAY OF ALFALFA MEAL

The particular alfalfa meal fed in this experiment was a brand manufactured in the West, and declared to contain at least 13 per cent protein. Since alfalfa meal is bulky and contains much fiber (about 30 per cent), it was fed at the 15 per cent level rather than at that of 30 per cent previously adopted for stuffs containing less than 30 per cent protein.

TABLE 2. *Oöcyst counts and weight gains for rats on 4 per cent dried yeast and 15 per cent alfalfa meal*

		(1) Reference series		(2) Test series		Ratio (2):(1)	
Trial	Rat number	Weight gain (gm.)	Oöcysts (10^6)	Weight gain (gm.)	Oöcysts (10^6)	Weight gains	Oöcyst counts
1	1	49	166	47	198		
	2	47	272	27	201		
	3	33	111	29	258		
	4	39	354	32	336		
	5	44	201	33	594		
	6	45	152	38	201		
	7	48	171	40	240		
	8	41	165	26	198		
	9	48	...	..	292		
	Mean	43.8	199	34	279.8	0.78	1.41
2	10	41	167	33	326		
	11	49	226	35	384		
	12	45	216	31	272		
	13	52	215	34	375		
	14	44	281	27	286		
	15	49	176	39	267		
	Mean	46.7	213.5	33.1	318.3	0.70	1.49
3	16	41	158	44	204		
	17	40	133	22	266		
	18	46	110	31	118		
	19	45	164	23	234		
	20	46	123	31	242		
	21	33	159	42	219		
	22	33	201	28	214		
	23	42	185	32	302		
	Mean	40.8	154.1	31.6	224.9	0.77	1.46
W						0.76	
F							1.45

The *W*-value of 0.76 as shown in table 2 indicates that growth on the alfalfa was only about three-fourths as good as on the 4 per cent yeast. This is somewhat surprising, since bright green alfalfa is known to contain considerable amounts of vitamins A, B, D, E, and G.

Despite restricted host-growth, the *F*-value was 1.45, a relatively high figure. Presumably, then, alfalfa contains liberal amounts of the hypothetical material or materials necessary for coccidium-growth.

ASSAY OF WHITE WHEAT FLOUR

In previous papers we reported that the *W*- and *F*-values for whole wheat were 1.628 and 0.922, respectively; for wheat bran, 1.73 and 1.91; for wheat middlings, 1.72 and 2.65. Hence, it seemed logical to test the white flour. The particular brand employed was a widely used, nationally advertised brand. It was fed at the 30 per cent level.

The results of the experiment summarized in table 3 yielded a *W*-value of 0.59 and an *F*-value of 0.52. Thus white wheat flour was far inferior to wheat, wheat bran, or wheat middlings in sustaining both host-growth and parasite-growth.

DISCUSSION AND SUMMARY

Since this completes, for the present at least, our program of assaying feeding stuffs for their "coccidium-growth-stimulating properties," it

TABLE 3. *Oöcyst counts and weight gains for rats on 4 percent dried yeast and 30 per cent white wheat flour*

Trial	Rat number	(1) Reference series		(2) Test series		Ratio (2): (1)	
		Weight gain (gm.)	Oöcysts (10^6)	Weight gain (gm.)	Oöcysts (10^6)	Weight gains	Oöcyst counts
1	1	29	109	21	73		
	2	39	97	21	77		
	3	33	129	19	67		
	Mean	33.7	111.7	20.3	72.3	0.60	0.65
2	4	30	306	22	102		
	5	36	248	21	87		
	6	27	151	23	67		
	7	36	164	32	105		
	8	43	125	22	110		
	9	25	89	19	40		
	Mean	32.8	108.3	23.1	85.1	0.70	0.79
3	10	65	103	32	27		
	11	47	155	30	56		
	12	59	115	21	37		
	13	58	158	28	42		
	14	63	159	35	31		
	15	51	152	31	67		
	16	61	265	27	28		
	17	50	132	28	37		
	Mean	56.8	154.9	29	40.6	0.51	0.26
W						0.59	
F							0.52

seems desirable to summarize briefly in semi-tabular form the stuffs tested in the order of their F-values (W-values in parenthesis):

Feeding Stuffs	F	(W)
Dried fish (whiting)	0.30	(0.53)
Meat scraps	0.31	(0.11)
Yellow corn	0.47	(1.092)
Oat hulls	0.49	(0.69)
Soy bean oil meal	0.51	(1.00)
White wheat flour	0.52	(0.59)
Hulled oats	0.59	(1.25)
Whole wheat	0.92	(1.63)
Linseed oil meal	0.94	(0.80)
Barley	0.98	(0.92)
Alfalfa meal	1.45	(0.76)
Rye	1.83	(1.50)
Wheat bran	1.91	(1.73)
Wheat flour middlings (gray shorts)	2.65	(1.73)

The summary depicts most strikingly the extent to which the number of oöcysts eliminated during experimental coccidium infection can be controlled through the composition of the ration.

In one of the previous papers (1937) it was emphasized that the extent of the parasite's *(Eimeria nieschulzi's)* reproduction in its host, as measured by oöcyst counts, is not simply a function of the rate of growth of the host. While, admittedly, the summary shows a certain degree of correlation between F-values and W-values, there are pertinent examples to support our previous contention: (1) Yellow corn, with a slightly higher W-value than barley, has an F-value less than half as great; (2) soy bean oil meal has a W-value of 1.00 which exceeds that of white wheat flour by about two-thirds, but the F-values are approximately the same; (3) there is little difference between the F-values for wheat and linseed oil meal, but the W-value of the former is twice that of the latter. A number of similar comparisons are apparent.

Likewise, the previous conclusion that the differences in oöcyst counts are not attributable to the amount of fiber in the supplement is further borne out; for example, alfalfa meal and oat hulls both contain about 30 per cent crude fiber, but the former's F-value is approximately three times the latter's. Numerous other examples could be given to support the contention regarding fiber.

At the present time no better hypothesis can be devised to explain the differential effects of the supplements on the coccidium population than the one previously stated by Becker and Morehouse (1937); namely, that there occurs in many feeding stuffs, often in association with vitamins B and G, a peculiar nutrient of the coccidium. It is our present intention to make this hypothesis the central problem of future investigation.

LITERATURE CITED

BECKER, E. R., and R. C. DERBYSHIRE
1937. Biological assay of feeding stuffs in a basal ration for coccidium-growth-promoting substance. I. Procedure, yellow corn meal, oats, oat hulls, wheat, linseed meal, meat scraps. Iowa State College Journal of Science, **11**: 311-322.

——— and ———
1938. *Ibid.* II. Barley, rye, wheat bran, wheat flour middlings, soy bean meal. *Ibid.*, **12**: 211-215.

——— and N. F. MOREHOUSE
1937. Modifying the quantitative character of coccidian infection through a dietary factor. Jour. Parasit., **23**: 153-162.

FACTORS AFFECTING THE MILK PRODUCTION OF SIMMENTHALER GRADE COWS UNDER THE PENKEEPING SYSTEM

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During the last 40 to 50 years the human population has increased rapidly in Brazil as in other tropical countries. The older cities are becoming more populous and new cities are arising. The urban population and the consumption of milk and milk products have increased together.

For several reasons, among which may be cited easy transportation, nearness to the markets, and natural conditions suitable for cattle raising, the southern and southeastern regions of the State of Minas Gerais are important sources of milk for the cities of Rio de Janeiro and São Paulo.

The native stock and Brahman breeds could not satisfy the demand for fresh milk. To solve this problem, the Federal (Ministerio) and State (Secretaria) Departments of Agriculture have imported animals of European dairy breeds, notably Holstein-Friesian, Jersey, Guernsey, Brown Swiss[2] and some dual-purpose cattle, such as Simmenthaler and others. The main objectives of these importations were to increase the number of purebred cattle, raised chiefly in the Experiment Stations, and to provide purebred bulls for lending to farmers for crossbreeding purposes.

The imported breeds have been crossed with native and Brahman cattle so that there are now found in these regions of the state crossbreds of various and partly unknown degrees of crossing.

The grading up method of breeding was recommended, as in other countries, but in general this process has failed, and the farmers are trying to develop a new type capable of thriving under the farm conditions of these regions. The practical experience of the breeders establishes that: (a) the Brahman and native cows are resistant to environmental conditions, but they are in general poor producers; (b) the purebred cow is not hardy enough under the actual farm conditions; (c) the infusion of imported blood upon native or Brahman stock improves the milking capacity; (d) as the amount of imported blood increases beyond a limit that is not definitely determined, hardiness decreases and the milk production falls along with it.

These facts have led the farmers to a practice called "blood refreshing" in an effort to control the losses such as weakness and low production from continually grading with purebred bulls. This is done in two ways (1) using high grade bulls on grade cows or (2) backcrossing the high grade cows to the original basic stock (as a rule Brahman).

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[1] The author made this study during a year spent in graduate work at the Iowa State College under the guidance of Dr. Jay L. Lush of the Animal Husbandry Department.

[2] The Brown Swiss is considered a dual-purpose breed in some countries, but in Minas Gerais, Brazil, this breed is raised almost exclusively for milk purposes.

The object of the present study was to identify and describe some of the factors which have an important effect on milk production, under the conditions of the "penkeeping system" usually known in Brazil as "sistema de retiros."

ENVIRONMENT

The environment plays an important part in dairy cattle production in Minas Gerais. Among the important environmental factors may be named climate, topography, pests and diseases, and management.

Minas Gerais is in the central part of Brazil, between the 15th and 20th parallels south, in the tropical belt. The temperature ranges from 0 to 40° C. Rhoad (20) found for four years an average of 254.1 10-hour days per year of tropical temperature. The relative humidity ranges from 57 per cent in August to 81 in December and January, and the annual average is 70 per cent[3]. In the municipality of Leopoldina the average precipitation was 1,156 mm. for 1934, 1935, and 1936. There are two definite seasons in the year, a rainy (from October to March) and a dry season (from April to September).

Among the important pests and diseases are: Ticks, "berne" *(Dermatobia hominis)*, flies, worms, foot-and-mouth disease, anthrax, black leg, pneumonia, tuberculosis, tick fever—some of which are equally dangerous to animals and men.

The system of management is one of the most important factors affecting cattle production in this part of Brazil. Rhoad (21), studying a dairy herd of the same region, referring to this management, wrote that "the cattle are kept on pasture the year round, fattening and giving an abundance of milk during the wet season, losing weight and drying up in milk flow during the winter or dry season. Making hay or preparing in any way for the winter season is not a general practice. For convenience of management the large herds are divided into 'retiros' or pens 20 to 40 head each and distributed over the farm in accordance with the particular farm conditions. Each pen has a simple shelter joined by a small corral. To this corral the cattle are brought twice a day, early in the morning to be milked and again in the afternoon to separate the calves from their dams. The calves remain separated until morning when one by one they are tied close by their dam to remain there until the cow is milked. The calf is then turned loose to get the strippings and to remain with its dam in the pasture until separated in the afternoon.

"There results from this method of management a very close psychological relationship between the cow and her calf. Many cows will not 'let down' their milk in the absence of the young. This is so characteristic of native and crossbred cows that when a calf dies its dam invariably goes dry shortly after."

However, the farm from which the present data were taken has a system of management a little different from that cited in the foregoing. The bulls are in the pastures with cows only from May 20 to January 20, so that the births occur in the dry season (approximately from March 1 to October 31). However, this practice is not rigorously followed, because the purebred bulls borrowed from the Department of Agriculture are

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[3] These figures refer to Viçosa, 640 m. above the sea level.

used at any time of the year. Apparently no special attempt has been made to breed the heifers at a definite age.

In this herd chopped sugar cane and, in recent years, some native grass silage are provided during the dry season. This feed is put in several mangers for all milking cows at the same time. It appears that this feeding, although not abundant, has maintained the cattle in a better condition than is usual on many farms and has even kept the milk production from declining during the winter months.

MATERIAL AND METHODS

For this analysis, the records of the Simmenthaler herd of the Fazenda Niagara, Leopoldina, Minas Gerais, were used. Mr. Gabriel Junqueira has continued the work of his father, started approximately 30 years ago, in an endeavor to establish a dairy herd for his own purposes. Although the predominant blood of the cattle is Simmenthaler, there is also some Brown Swiss blood from several grade cows, purchased on neighboring farms for their special dairy qualities. This breeder still had to use a purebred Holstein bull in 1933 because of a foot-and-mouth disease attack that made the Simmenthaler bulls completely unserviceable.

During these 30 years bulls and cows of several degrees of blood from one-eighth Simmenthaler to purebred were used. For many cows the amount of European blood was known.

Records of production during 19 years have been considered in this study. Only records of cows which had already left the herd were included. The herd records are kept carefully and give the day of the cow's birth, length of the lactation period, total milk yield and some remarks, such as death of calf, occurrence of foot-and-mouth disease and disposal of the cow.

The individual production was measured every fifteen days on the first and sixteenth of each month, using a "specially devised container that eliminated the voluminous effect of foam, produced by pouring warm milk into the container"[4]. The milk for the calf was always left in the cow's udder. The production of every cow was recorded separately, and at the end of the lactation the sum of the bi-monthly records $\times$ 15 gave the total milk yield for the lactation.

The figures for the total milk yield and lactation period for every lactation were simply copied from the farm book without any change, as the data were correct from the practical point of view. In many cases the length of lactation period (from calving to weaning) was really longer than that recorded, but this did not affect the accuracy, for the cow was then producing so little that the milk was not measured. The weaning was thus delayed for 10 or more days to permit the cow to go dry naturally without udder troubles or a sudden change in the calf's feeding.

The length of service period was calculated for every lactation by subtracting 283 days from the interval between the dates of two successive calvings.

The interval between the date of weaning and the next calving is considered the dry period.

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[4] Rhoad in his work (21) described this container which is generally used on the farms of that region.

LITERATURE

Several tropical and sub-tropical countries have tried the so-called fine breeds and some results have been reported.

Hammond (8) emphasizes that for transferring animals from temperate to tropical regions, not only the resistance to diseases and adaptability to conditions of nutrition should be remembered, but also the ability to withstand high temperature.

Bisschop (1) from South Africa has indicated that a certain limit to the grading up policy is necessary to prevent the failure of imported breeds.

Tarantino (24) reported that in Italian Somaliland the reddish cattle are well adapted to work under the most arduous conditions. The native cows are poor producers, but the Sardinian Brown crossbreds are better milkers.

Bunting and March (2) report that no indigenous domesticated cattle are found in Malaya, the herd at Serdang consisting of imported Montgomeries, Friesians and some crossbreds, and Jerseys. The imported and locally bred Indian cattle have thrived, but the European breeds have not given satisfactory results.

Hammond (9) attributes the failure of European breeds in Jamaica and Trinidad to tick fever, long hair, white skin, improper feeding and possibly climate. He reports the breeds varied in the extent to which the degenerative changes occurred, the smaller breeds being less affected than the larger ones.

Edwards (5), working also with material from Jamaica, points out the value of a proper genetic constitution of cattle for tropical conditions. He shows that a well bred Guernsey bull reduced the milk yield 400 pounds, while a pure Montgomery bull increased the yield 970 pounds. It is emphasized in this work that the improvement in constitution was more remarkable than in milk production. He concludes from his study that "the occurrence of the highest average yields among the grades possessing one thirty-second to one quarter Zebu blood coupled with a lower percentage of failures in these grades, points to their being the most satisfactory medium for the development of a new breed suited to the environment."

Kartha (13), comparing the efficiency of the Indian cow, the half-bred cow and the buffalo as producers of milk and butter-fat, concludes that under existing conditions the buffalo is more economic for the villager.

In Minas Gerais, Brazil, Carneiro and Rhoad (3) found that "purebred Holstein calves from imported dams showed a decrease in the rate of growth relative to the normal growth of the breed. This growth check occurs from the fourth month on and is accentuated after the sixth month. Environmental factors other than nutrition and care are responsible for the sub-normal development. Under similar conditions crossbred calves develop normally."

Rhoad (21), working with cattle in Minas Gerais, Brazil, found no distinction between yields of first, second, and third lactations under the penkeeping conditions. His work showed also a pronounced effect of the dry season on milk production and on the reproductive capacity of the animals. More recently Rhoad (20) has shown the influence of environmental temperature on the respiratory rhythm of dairy cattle in the

tropics (M. Gerais), as "at 36° C. the European cattle apparently reached their maximum efforts of physical regulation through the lungs. This, however, was not indicated in the respiration curve for Zebu cattle."

R. Joviano (12) reports results (1919-1932) in the Federal Experiment Farm of Pedro Leopoldo, M. Gerais, with some imported breeds. In 12 years 77 Brown Swiss calves were born, of which 20 died. The year of 1931 marks the smallest percentage of calf deaths—30 per cent. Regarding the Simmenthaler, in 6 years 47 individuals were introduced to the farm. During this time 26 calves were born, of which 12 died; of the mature individuals 32 died. The Farm received the first lot of Holsteins in 1928, and others in 1929 and 1930, making a total of 45 head, of which only 25 were still alive in 1932.

Rhoad (22) showed a good increase in the milk yield by changing high-grade Holstein cows from the penkeeping system to a modern system of management.

The present results in general agree with those found by Edwards (5) in Jamaica and by Rhoad (21) in Brazil. The likeness of objectives and environment led the writer to consider the two previous studies as points of reference in analyzing and interpreting the present results.

RESULTS

On most points the data are summarized in tables according to Fisher's (6) method of "Analysis of Variance." The figures following the ± sign refer to standard error throughout this study.

AGE OF THE HEIFERS AT FIRST CALVING

It is known that cattle in the warm climates require more time to mature than in temperate regions. Edwards (5), working with material from Jamaica, found that the heifers usually calve for the first time when about 3.5-4 years old. They may be considered late in this respect since Plum and Lush (17) found a mean age at first freshening of the heifers in the Iowa Cow Testing Associations of 27.1 months.

For only 282 heifers were the ages at first calving known definitely; the mean age of these was 38.7 ± .3 months. The standard deviation was 4.9 months.

To find whether there was any relation between the time of year when the heifer was born and her age at first calving, the heifers were sorted according to the month of birth and for each of the 12 resulting groups the ages at first calving were studied. The results are shown in table 1.

TABLE 1. *Month of birth of the heifers and their age at first calving*

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
No. in the month..	4	17	57	49	35	26	22	11	20	22	11	8	282
Mean age in months.	39.7	40.0	38.1	38.8	37.9	38.2	38.1	40.7	38.4	39.2	40.5	38.5	38.7

Table 2 shows the analysis of variance at age of first calving between and within groups sorted according to the month in which they were born.

TABLE 2. *Variance of age at first calving as related to time of year when born*

	Degrees of freedom	Sum of squares	Mean square
Total	281	6,834	24.3
Between months	11	177	16.1
Within months	270	6,657	24.6

There is no indication that the month of birth of the heifers has an influence on their age at first calving.

Table 3 shows that the distribution of calving throughout the year is about the same for heifers as for the cows. Apparently no attempt was made to have the heifers calve for the first time at a definite age.

TABLE 3. *Percentage of heifers and cows calving at various months of the year*

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Heifers	1.0	5.3	30.0	15.3	10.6	8.0	8.0	3.6	7.3	8.0	2.0	0.6
Other cows	4.5	6.5	24.6	13.7	10.8	9.4	9.3	5.2	4.5	5.2	2.3	3.6

THE PRODUCTIVE LIFE OF THE COWS

In many countries, even those where the dairy industry is really advanced, the length of productive life is an object of discussion or is not well known at the present time. Lush and Lacy (15) found that the average productive life (from the first to the last calving) among registered cows of the dairy breeds in the United States is 3.5 years and that 28 per cent are replaced every year.

Practically nothing is known about this under the penkeeping conditions. In the present study we tried to clarify this, despite the small number available and the limitation of the data to only one farm in Minas Gerais.

The date of weaning in the last normal lactation was considered the end of the productive life, because in most cases the death of the cow was not recorded. Usually the cows were sold to the butcher or died a short time after weaning. The results were:

Number of cows	278
Mean age at which the cows were culled (date of weaning in the last normal lactation)	112.5 months
Standard deviation	38.0 "
Productive life (from first calving to the end of the last normal lactation)	73.8 "

The productive life of the cows seems long, when those found in the United States and Britain (23) are considered.

Because it was thought that the age at first calving might affect the productive life that relation was explored. The figures in table 4 show no indication that age at first calving affected the length of the productive life. The correlation was not significant, $r = -.051$.

TABLE 4. *Variation in length of productive life*

	Degrees of freedom	Sum of squares	Mean square
Total	277	400,275	1,445
Between ages at first calving ..	25	26,908	1,076
Within ages at first calving	252	373,367	1,482

MILK YIELD

The average milk yield for 1,196 lactations studied was 1,221 ± 13 liters, 65 liters higher than that found by Rhoad (21). This difference is small but statistically significant. However, this higher yield was produced in a longer lactation period.

The yields ranged from 275 to 3,575 liters, but a large part of this wide variability was caused by the variation in length of lactation period.

LACTATION PERIOD

The mean length of lactation period was 321 ± 2 days, but the range was extremely high, extending from 108 to 693 days. As will be seen later, the lactation period is one of the most important factors affecting the total milk yield.

SERVICE PERIOD

Rhoad (21) emphasized the importance of the service period for determining the reproductive ability of the dairy herd under the pen-keeping management. The long service periods of the Brahman cows have been noted by several workers and this fact may have influenced results obtained in the present study, since the type of cows showed the occurrence of Zebu blood. Detailed information regarding the breeding of the original foundation cows was not available.

The average service period is 197 ± 4 days, which is higher than that found by Rhoad (21) in the same region and under similar management, but is not extreme and is close to that found by Edwards (5) for Jerseys at Jamaica—196 days. The range was 892 and the standard deviation was 143 days.

DRY PERIOD

The dry period averaged 109 ± 2 days, and the variation in this was not extreme. The standard deviation was 61 days.

TABLE 5. *Summary of the results for milk yield, lactation period, service period, and dry period*

	Number	Mean	Standard deviation
Milk yield	1,196	1,221 ± 13 liters	443 liters
Lactation period	1,196	321 ± 2 days	87 days
Service period	1,060	197 ± 4 days	143 days
Dry period	1,056	109 ± 2 days	61 days

MONTH OF CALVING AND YIELD

Edwards (5), studying records of production from Jamaica, found that the yields of cows calving during the dry season were higher than those of cows calving in the wet season.

Rhoad (21) working with cattle of Leopoldina, M. Gerais, found similar results, that is, a relation between month of calving and milk yield. Cows calved in May, June, and July (dry season) had a higher milk production and a correspondingly longer period of lactation than those calved in the rainy season. He believed this fact was "due to the natural good fleshing of dry pregnant cows as they enter and go through the drought season. Cows of this group have more body reserves for carrying them through the drought and will produce a normal quantity of milk for the dry months. About the time their body reserves become exhausted the rains come, furnishing an increasing supply of food and thus sustain production for 30 to 40 days longer than normal."

In the present study, 1,193 lactations were grouped according to the month of calving in order to find out if this factor has some effect on milk yield. The average production for the cows in each such group is shown in figure 1. The plus and minus deviations from the general mean are irregular and do not indicate clearly any effect of the season of freshening.

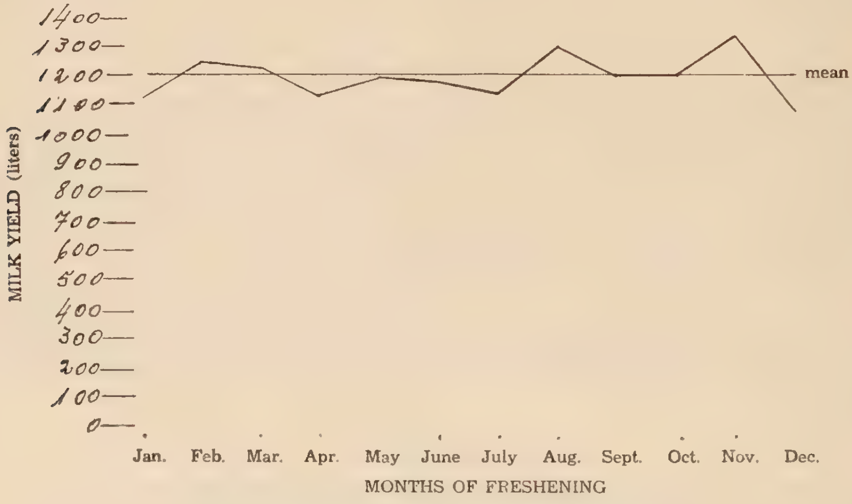

FIG. 1. Yield as related to month of freshening

TABLE 6. *Analysis of variance of the influence of month of calving on milk yield*
(Milk yield is expressed in units of 50 liters)

	Degrees of freedom	Sum of squares	Mean square
Total	1,192	93,932	79
Between months	11	1,253	114
Within months	1,181	92,679	78

The analysis of variance did show a slightly larger mean square between months than within months, but the difference was not statistically significant.

The difference between the findings recorded in table 6 and those of Rhoad (21) possibly may be attributed to the fact that no supplementary feed was available during the dry months, while in the present data a small amount of supplementary feed was given to the herd during the dry season. This is only a tentative explanation.

AGE OF THE COWS AND MILK YIELD

Usually it is established that in temperate climates the highest milk yield occurs when cows are 7 to 9 years old. In the tropics the time of highest milk yield is not established. Edwards (5) found the maximum production at the fifth lactation, when he compared "the average yield of first calvers with the average of the same group of animals in their second lactation, and so on." However, when he compared the lactations by ages without regard to whether the same cows were represented, there was a slight increase in production to the fourth lactation. He explained this in part by supposing that many of the best cows lacked the proper constitution to endure tropical conditions and were incapable of producing normally for more than one or two lactations.

Under penkeeping conditions Rhoad (21) found little difference in milk production of the first, second, and third lactations. He attributed this to the feeding conditions and lack of culling poor producers.

In the present study 1,085 lactations were grouped according to the age of the cow at freshening. The results are shown in table 7 and figure 2.

TABLE 7. *Analysis of variance of age on milk yield*
(Milk yield expressed in units of 50 liters)

	Degrees of freedom	Sum of squares	Mean square
Total	1,084	77,562	72
Between ages	25	4,202	168
Within ages	1,059	73,360	69

The influence of age on milk yield is significant statistically but not important practically, for the mean square is reduced only about 3 per cent by holding age constant.

Two facts in figure 2 are worthy of comment (a) the remarkable fall of the yield curve at 38.5, 44.5, and 50.5 months of age and (b) the approximately constant production at the ages from 56.5 to 128.5 months. The irregularity at ages past 134.5 months is mainly because of the few lactations at these ages.

Variations in length of the lactation period are at least partially responsible for this fall in yield at the younger ages.

When the average of the total yield was found for every age group and divided by the corresponding average number of days of lactation, then this decrease in yield at the younger ages disappeared and the daily milk yield rose steadily to 56.5 months of age, as shown in figure 3.

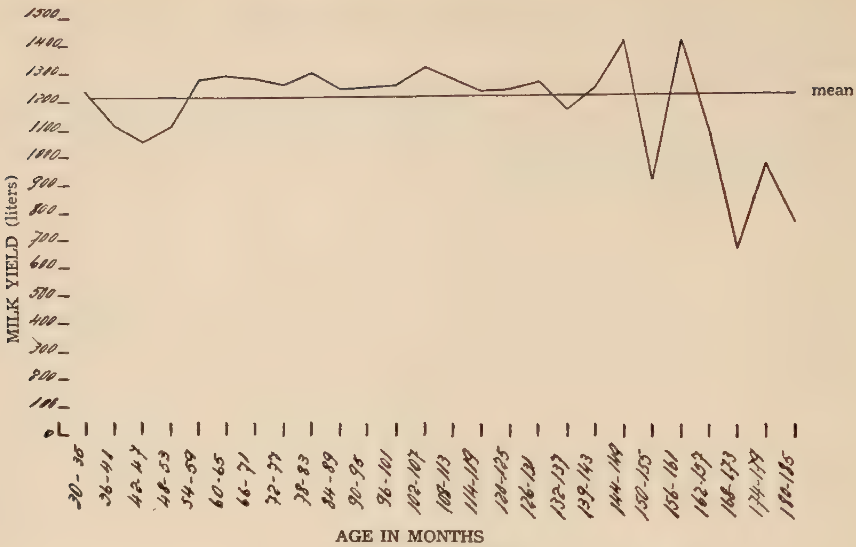

FIG. 2. Gross yield as dependent on age of cow at freshening

THE INFLUENCE OF LENGTH OF LACTATION PERIOD ON YIELD

The highest yields were in general those made during long lactations whereas the low tended to be from short lactation periods. The analysis of variance (table 8) showed a highly significant result. Variance in yields

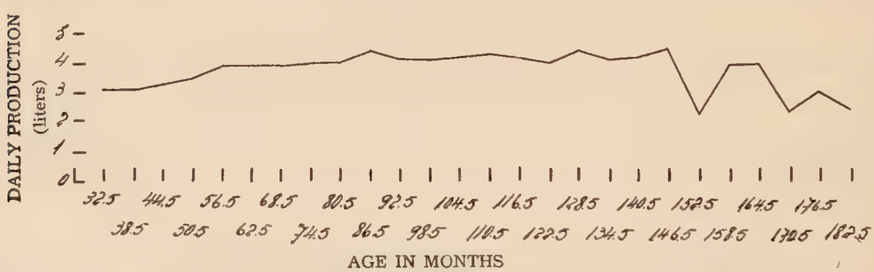

FIG. 3. Average yield per day in milk for cows freshening at various ages

TABLE 8. *Analysis of variance of the influence of lactation length on milk yield* (Milk yield is expressed in units of 50 liters)

	Degrees of freedom	Sum of squares	Mean square
Total	1,195	94,010	79
Between groups with different lengths of lactation period ...	37	46,119	1,246
Within groups having the same lactation period	1,158	47,891	41

was reduced 47.4 per cent when lactation period was held constant. The correlation between yield and lactation length was +.69. Since the square of this correlation coefficient is almost identical with the fraction of the variance which disappears when length of lactation is held constant, the relation is practically linear.

This linearity is reasonable on the basis that the management—one milking daily, the natural calf feeding (suckling its dam), feeding conditions of the dairy cows—keeps the daily milk yield at a relatively constant level.

Figure 4 shows the mean yields for each length of lactation and the regression of milk yield on length of lactation period. From this regression appropriate factors for correcting milk yield to a standard lactation length of 305 days have been tabulated. They are shown in table 9. The regression equation is:

$$\text{Estimated yield} = 1{,}166.67 + 3.52\ (\text{L.P.} - 305).$$
$$= 93.07 + 3.52\ \text{L.P.}$$

TABLE 9. *Factors for correcting milk yield to a standard lactation length of 305 days*

Lactation period	Correction factor	Lactation period	Correction factor	Lactation period	Correction factor	Lactation period	Correction factor
103	2.562	233	1.278	358	.862	498	.644
108	2.467	238	1.254	363	.851	493	.638
113	2.378	243	1.230	368	.840	498	.632
118	2.296	248	1.208	373	.830	503	.626
123	2.219	253	1.186	378	.819	508	.620
128	2.147	258	1.165	383	.809	513	.614
133	2.080	263	1.145	388	.800	518	.609
138	2.016	268	1.125	393	.790	523	.603
143	1.957	273	1.107	398	.781	528	.598
148	1.901	278	1.089	403	.772	533	.592
153	1.848	283	1.071	408	.763	538	.587
158	1.798	288	1.054	413	.754	543	.582
163	1.750	293	1.038	418	.746	548	.577
168	1.705	298	1.022	423	.737	553	.572
173	1.660	303	1.006	428	.729	558	.567
178	1.622	305	1.000	433	.721	563	.562
183	1.583	308	.991	438	.714	568	.557
188	1.546	313	.976	443	.706	573	.553
193	1.511	318	.962	448	.699	578	.548
198	1.477	323	.949	453	.691		
203	1.445	328	.935	458	.684		
208	1.414	333	.922	463	.677		
213	1.384	338	.909	468	.670		
218	1.356	343	.897	473	.664		
223	1.329	348	.885	478	.657		
228	1.303	353	.874	483	.651		

The 305-day corrected yields in liters are plotted against age in months in figure 5. The shape of the curve follows somewhat closely that found by Edwards (5) for all the data on lactations of dairy cattle in Jamaica. A glance at figure 5 shows that the shape of the curve for milk

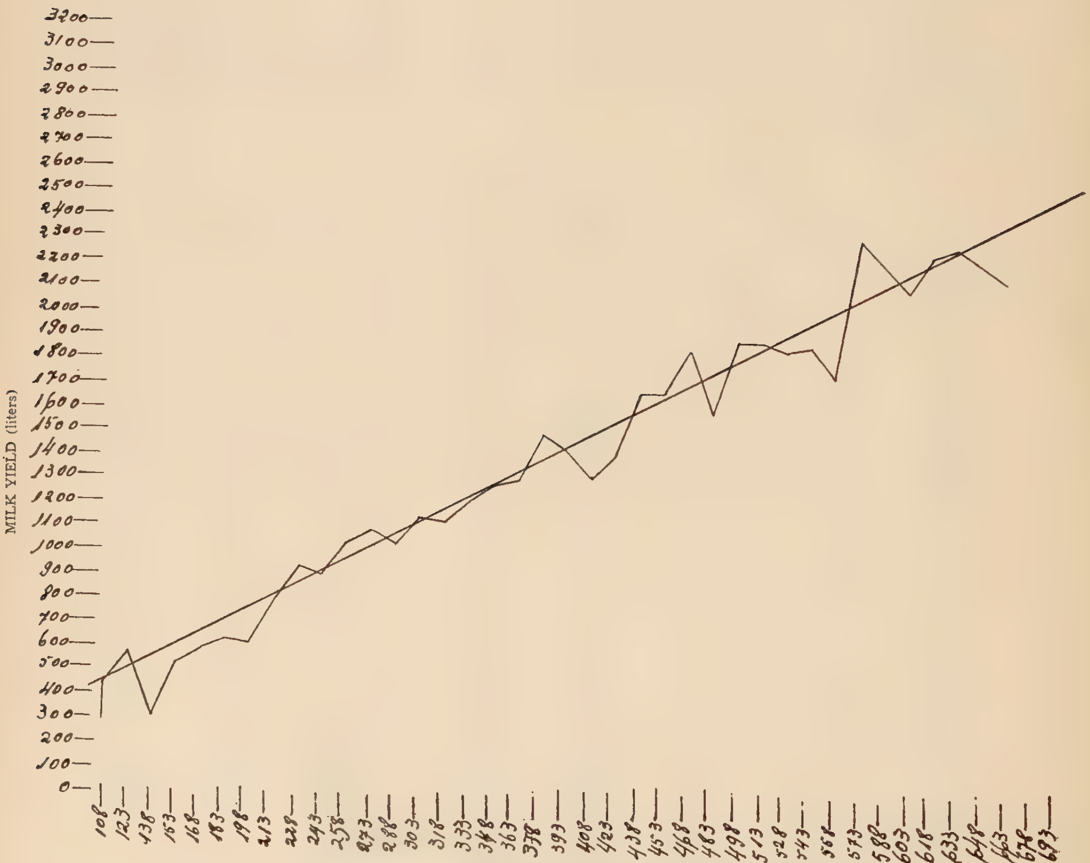

FIG. 4. Regression of milk yield on length of lactation period.

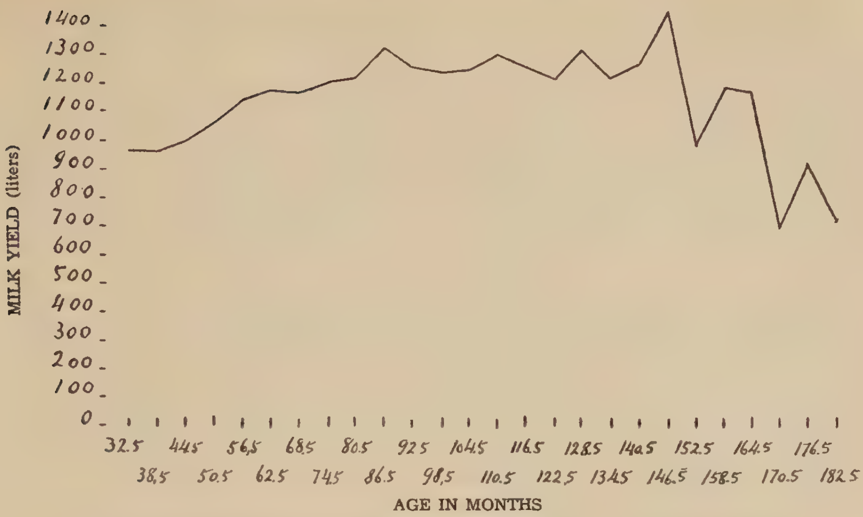

FIG. 5. Age at freshening and yields standardized to a length of 305 days

yield as dependent on age is not very different from that of the temperate regions.

THE EFFECT OF LENGTH OF SERVICE PERIOD ON YIELD

TABLE 10. *Analysis of variance*
(Yield expressed in units of 50 liters)

	Degrees of freedom	Sum of squares	Mean square
Total	1,059	80,368	76
Between groups of service period	34	17,142	504
Within groups of service period	1,025	63,226	62

Length of service period was found to exert a significant influence on yield. Variance in yield was reduced 18.4 per cent when service period was held constant (table 10). This agrees with Edwards' data (5), where the service period and age appeared to be the most important environmental factors (except feeding) affecting yield. Kartha (13), working with cattle in India, found a positive correlation between yield per lactation and length of service period, but lactation yield also was correlated with lactation period.

In this study partial correlations were used as one way to separate the influence of lactation period from that of service period on milk production per lactation. The formula is:

$$r_{12.3} = \frac{r_{12} - (r_{13}\, r_{23})}{\sqrt{(1 - r^2_{13})\,(1 - r^2_{23})}}, \text{ where}$$

1 = yield; 2 = lactation period; 3 = service period.

The partial correlation between yield and lactation period, service period being constant, was +.66, which is only a little less than the primary correlation, +.69.

On the other hand, holding the lactation period constant brought out a negative correlation between yield and service period, —.23, which is distinctly different from the primary correlation, +.35. Among cows having the same length of lactation period, those with the longest service periods had the lowest yields. The multiple regression equation for predicting yield from both lactation period (LP) and service period (SP) is:

$$Y = 4.33\,LP - .71\,SP - 27.$$

When both are thus taken into account simultaneously an additional day in the lactation period means an average increase of 4.33 liters in the milk yield while an additional day in the service period means an average decrease of .71 liters in the milk yield. Length of service period has little practical influence on yield except through its connection with length of lactation period which does have an important effect. (Curvilinearity, if it exists, might make these average relations noticeably untrue at certain special lengths of service period.)

THE EFFECT OF AGE ON LENGTH OF LACTATION PERIOD

TABLE 11. *Analysis of variance in length of lactation period*
(Lactation period expressed in units of 15 days)

	Degrees of freedom	Sum of squares	Mean square
Total	1,084	36,501	34
Between ages	25	3,495	140
Within ages	1,059	33,006	31

Although the farmer's control may be the most important cause of lactation period length, its non-uniformity for the several ages suggested that age might have some influence on it. Table 11 shows that the mean square within ages (31) is 8.8 per cent less than the general mean square (34) for lactation period length in the whole population. The correlation coefficient between age and lactation period was —.22, which shows a decrease (although not a very large one) in the length of L.P. as age increases. All the cows included in the first two ages (from 30-41 months) were first calvers. Also in the 44.5 months group (from 42-47), 43 of 59 individuals were first calvers.

A highly significant difference of 36 days existed between the average lactation periods of the first two age-groups (32.5 and 38.5) in table 12. Whether this was due to something in the management is not known. The work of Eckles (4) shows that lactating heifers grow less than pregnant heifers of the same age and breed. This effect was decidedly accentuated when early calving was combined with light feeding. In the present case, possibly milk production, poor feeding conditions, and perhaps effort to grow, did not permit the young heifer to come in heat again quickly, so that the length of lactation period was indirectly increased through that of service period. The heifers calving at 32.5 months had an average service period of 214.5 days, whereas those calving at 38.5 months had only 171.8

TABLE 12. *Length of lactation and daily yield as dependent on age*

Age in months	Frequency	Lactation period	Daily milk yield in liters	Age in months	Frequency	Lactation period	Daily milk yield in liters
32.5	67	393.0	3.1	110.5	50	295.5	4.3
38.5	95	357.0	3.1	116.5	36	297.0	4.2
44.5	59	325.5	3.3	122.5	46	309.0	4.0
50.5	74	318.0	3.5	128.5	28	291.0	4.4
56.5	72	328.5	3.9	134.5	34	286.5	4.1
62.5	70	328.5	3.9	140.5	22	295.5	4.2
68.5	59	328.5	3.9	146.5	16	315.0	4.5
74.5	70	318.0	4.0	152.5	11	277.5	2.3
80.5	57	316.5	4.1	158.5	10	361.5	3.9
86.5	42	282.0	4.4	164.5	4	277.5	3.9
92.5	54	300.0	4.2	170.5	7	288.0	2.3
98.5	52	309.0	4.1	176.5	1	318.0	3.1
104.5	48	316.5	4.2	182.5	1	318.0	2.4

days, or 42.7 days less. The difference between mean service periods was not significant ($t = 1.70$ with 145 degrees of freedom), but the difference did exist.

THE INFLUENCE OF MONTH OF CALVING ON THE LENGTH OF SUBSEQUENT SERVICE PERIOD

Rhoad (21) mentioned two facts which he believed were responsible for the length of the service period: the number of unbred cows and the state of nutrition of the herd.

In the present study 1,060 service periods were sorted according to the month in which the preceding calving occurred. The analysis of variance (table 13) showed no significant influence of the month of calving on the length of the subsequent service period.

TABLE 13. *Analysis of variance in service period as related to month of preceding calving*

(Service period expressed in units of 25 days)

	Degrees of freedom	Sum of squares	Mean square
Total	1,059	34,757	32.8
Between months	11	548	49.8
Within months	1,048	34,209	32.6

However, when the average service period was calculated for every month, all plus deviations from the general mean were grouped in seven adjacent months, while all minus deviations were in the other five consecutive months (figure 6). When these were tested against each other, the difference was significant, thus indicating some effect of season of calving, although not an important effect practically.

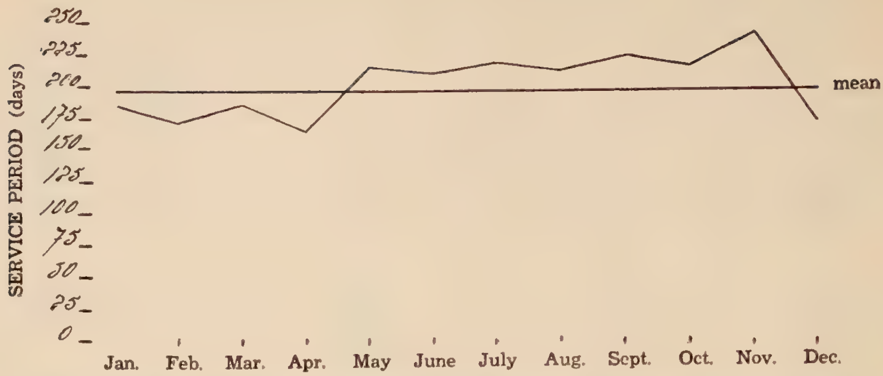

FIG. 6. Length of service period as dependent on the month when the preceding calving occurred

THE INFLUENCE OF HEREDITY

The value of breeding purebred bulls to scrub cows has been pointed out by several writers and the subject is emphasized in the books on Animal Breeding and Genetics (Winters, 1930) (Rice, 1934) (Babcock and Clausen, 1927). In many instances the increase in the second generation over the scrub is more than 100 per cent.

The value of breeding with purebred bulls in the tropics has been reported by many workers, such as Hammond (9), Metivier (16), Edwards (5), Rhoad and others. But, at least as far as the penkeeping system is concerned, no effort has been made yet to find out how much of the variation in milk yield is due to inheritance.

In the United States, Gowen (7) found that 50-70 per cent of the variation in milk yield is due to inheritance but that rested in part on assuming that none of the observed daughter-dam correlation came from environment common to each such pair. Plum (18) found that in fat production in a Jersey herd "sire" was responsible for 11.6 per cent of the variance, "year" for 9.8 per cent, "sire" and "year" jointly for 9.2, and things other than sire and environment for 69.4 per cent. Harris, Lush, and Schultz (11) report for lactation records an average intra-herd correlation between records of the same cow of .325, showing the tendency of an individual cow to repeat her yield, lactation after lactation. Plum (19) studying differences in fat production in Iowa Cow Testing Associations found the intra-herd correlation between one record of a cow and another record of the same cow to be .40.

In the present data 148 cows, for which it was possible to obtain first and second records of milk yield, were studied to learn to what extent the first two records of the same cow are repeatable, under the penkeeping management. The milk yields were corrected only for lactation period, as it was the only factor of practical importance.

A correlation of +.44 was found. This result means that permanent factors, including inheritance and permanent environmental effects, are responsible for 44 per cent of the variance in milk yield, corrected to a standard length of lactation period. The hereditary part properly thus is

responsible for something less than 44 per cent of the variance in milk production in this herd. This result is similar to those found in the United States.

THE AMOUNT OF IMPORTED BLOOD

As was said in a preceding section of this study, some information could be obtained on the amount of imported blood in various animals in this herd. Although these figures were not entirely accurate, the writer personally believes they are not far from the truth. The amount of imported blood was not high, despite the relatively long time spent already in building up this herd. Information obtained from the farm indicates that some difficulties have been met in raising the cattle with a high percentage of imported blood. This might be one reason there were more animals with a little foreign blood than with a large amount. The latter may have tended to be eliminated naturally on account of their lack of a proper constitution to thrive under the existing conditions.

As in the present case the number of lactation records varied between the groups with different percentages of exogenous blood and even among the cows of the same blood, it was decided to find each cow's most probable ability on the basis of the average of all records. The formula given by Lush (14) was used:

Most probable producing ability of the cow =

$$\frac{nr}{1-r+nr} \text{ x (her average record)} + \frac{1-r}{1-r+nr} \text{ x (the herd average)},$$

where n is the number of cow's records and r is the coefficient of correlation between records of the same cow ($r = .4$ in the present case). The results are shown in table 14.

TABLE 14. *Productivity and longevity of cows grouped according to the amount of their inheritance coming from recently imported dairy breeds*

Amount of imported blood	Frequency	Cow's ability	Deviations from the herd average 305 days corrected milk yield: 1,165 liters	Lifetime in months	Deviations from the herd average lifetime: 113 months
1/4	49	1,171	+ 6	114	+ 1
3/8	10	1,097	— 68	101	— 12
1/2	77	1,175	+ 10	126	+ 13
5/8	11	1,120	— 45	88	— 25
3/4	9	1,086	— 79	106	— 7
Purebred	8	1,109	— 56	105	— 8
Total	164				

No definite or steady decrease in production follows the increase in the percentage of imported blood, although there may be some

tendency of that kind. Also the length of the cows' lifetime roughly parallels the average yield of milk. This leads one to think of some real influence, which affects alike the ability to withstand the environment and to produce. The analysis of variance shows a significant influence of amount of imported blood on the lifetime of the cows (table 15). The variance within groups having the same amount of imported blood is 10.7 per cent less than the general variance. But the correlation between amount of imported blood and length of lifetime is only —.06, which is not significant, though it indicates that the lifetime becomes a little shorter as the amount of exogenous blood increases. The closer relation shown by the analysis of variance than by the correlation coefficient

TABLE 15. *Analysis of variance in length of lifetime (in months), grouped according to fraction of exogenous blood*

	Degrees of freedom	Sum of squares	Mean square
Total	164	5,018	31
Between groups	5	588	181
Within groups	159	4,430	28

suggests that the relation is curvilinear. Whether the higher yield and longevity found for one quarter and one half bred animals were a consequence of heterosis could not be determined for their pedigrees were not explicit enough to separate those which might be expected to show heterosis from those which would not. Heterosis may be one of the causes, but probably not the single one, as Hammond (10) pointed out, referring to the cross between Zebu and European cattle: "This is not merely a matter of hybrid vigor, for different European breeds crossed together in the tropics give no better results than those breeds when pure."

SUMMARY AND CONCLUSIONS

1. The mean age of the heifers at freshening was 38.7 ± .3 months. No relation was found between the month of the year in which the heifers were born and their age at first calving. The distribution of calvings throughout the year was about the same for heifers as for the rest of the cows.

2. The mean age at which the cows ended their last lactations before they left the herd was 112.5 ± 2 months. The relation between age at first calving and the productive life of the cows was negative, but insignificantly so.

3. The month of calving showed no significant influence on milk yield. The plus and minus deviations from the general mean were irregular, not indicating clearly any effect of season of freshening on milk production.

4. The influence of age of the cows on the actual milk yields was statistically significant but not important practically. The variance was only 3 per cent less within groups of cows of the same age than it was among all cows.

5. The length of lactation period had the most pronounced effect on

total milk yield. The variance in yields was reduced 47.4 per cent when lactation period was held constant. The correlation between yield and lactation length was +.69, showing a practically linear relation.

6. The effect of service period on milk yield was statistically significant and the variance in yield was reduced 18.4 per cent when service period was held constant. The correlation between them was +.35. However, when the influence of service period was separated from that of lactation period, the partial correlation between service period and yield, length of lactation being constant, was —.23, showing little practical influence of service period on milk production.

7. The age of the cow had a significant effect on the length of lactation period, the correlation being —.22 showing a decrease in the length of L.P. as age increased. A significant difference of 36 days existed between the average lactation periods of the two groups calving at ages of 32.5 and 38.5 months.

8. When the average service period was calculated for the groups which calved in different months, all plus deviations from the general mean were in seven adjacent months (March to November), while all minus deviations were in the other five consecutive months. The difference of these two groups was significant, but the effect was not important practically.

9. In this study 44 per cent of the variance in milk yields, standardized to a lactation length of 305 days, were due to permanent differences between individual cows, including inheritance and permanent environmental effects.

10. No regular decrease in production paralleled the increase in the percentage of imported blood but there was some indication of such a tendency, probably curvilinear, either because of heterosis or because the maximum yield comes from an intermediate percentage of imported blood. The influence of amount of imported blood on the cows' lifetime was significant, when analyzed by variance wtihin and between classes, but an insignificant correlation (—.06) shows that the relation is curvilinear.

REFERENCES

1. Bisschop, J. H. R.
1934. Environment: Its relation to cattle-breeding under veld condition. Farming in South Africa, 9:91.

2. Bunting, B., and T. D. March
1934. The Government Dairy Farm, Serdang. Malay. Agric. J., 22:216-227. (Abstracted in An. Breeding Abstracts, 3:362.)

3. Carneiro, G. G., and A. O. Rhoad
1936. The development of calves in the tropics. Tropical Agriculture, 13: 177-180.

4. Eckles, C. H.
1937. Dairy cattle and milk production. Macmillan Co., New York.

5. Edwards, J.
1932. Breeding for milk production in the tropics. Jour. Dairy Res., 3:281-293.

6. Fisher, R. A.
1936. Statistical methods for research workers. Sixth edition. Oliver and Boyd, Edinburgh and London.

7. Gowen, John W.
1934. The influence of inheritance and environment on the milk production and butterfat percentage of Jersey cattle. Jour. Agr. Res., 49: 433-465.

8. Hammond, John
1936. Environmental conditions and livestock breeding. Problems of Animal Husbandry No. 8. U.S.S.R.

9. ———.
1932. Report on cattle breeding in Jamaica and Trinidad. Publication No. 58 of the Empire Marketing Board.

10. ———.
1931. Tropical dairying problems. Tropical Agriculture, 8: 311-315.

11. Harris, G. M., Jay L. Lush, and E. N. Schultz
1934. Progress report on comparison of lactation and yearly records. Jour. Dairy Sci., 17: 737-742.

12. Joviano, R.
1919-1932. Fazenda Modelo de Criacao de Pedro Leopoldo. Boletim do Ministerio da Agricultura, Industria e Commercio.

13. Kartha, K. P. R.
1934. A note on the comparative economic efficiency of the Indian cow, the half-breed cow and the buffalo as producers of milk and butter fat. Agr. and Live Stock in India., 4: 605.

14. Lush, Jay L.
1937. Animal Breeding Plans. Collegiate Press, Inc., Ames, Iowa, pp. 125-131.

15. Lush, Jay L., and M. D. Lacy
1932. The ages of breeding cattle and the possibilities of using proven sires. Iowa Agr. Exp. Sta. Bul. 290.

16. Metivier, H. V.
1928. Tropical dairy cattle. Tropical Agriculture, 5: 131 and 188.

17. Plum, Mogens, and Jay L. Lush
1934. Freshening ages of purebred cows in Iowa Cow Testing Associations. Jour. Dairy Sci., 17: 625-638.

18. Plum, Mogens
1933. Production in a large Jersey herd as affected by sires, dams and yearly variations. Proc. Amer. Soc. An. Prod., pp. 53-57.

19. ———.
1935. Causes of differences in butterfat production of cows in Iowa Cow Testing Associations. Jour. Dairy Sci., 18: 811-825.

20. Rhoad, A. O.
1936. The influence of environmental temperature on the respiratory rhythm of dairy cattle in the tropics. Jour. Agr. Science, 26: 36-44.

21. ———.
1935. Production of Brazilian dairy cattle under penkeeping system. Zeitschr. Zücht. Reihe B, Tierzücht. u. Züchtungsbiol., 33: 105-118.

22. ———.
1933. Principios basicos para melhoramento do gado leiteiro nos tropicos. Minas Gerais, Brazil. Secretaria da agricultura. Bol. Agr. Zootech. Vet., 6: 661-671.

23. Smith, A. D. Buchanan, and O. J. Robison
1931. The average ages of cows and bulls in six breeds of cattle. Jour. Agr. Sci., 21: 136-149.

24. Tarantino, G. B.
1934. L'industria zootecnica nella Somalia italiana. Critica Zootec., 11: 368-373. (Abstracted in An. Breeding Abstracts, 3: 777.)

A GAS-TYPE X-RAY TUBE

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Received May 8, 1939

The x-ray tube described in this paper is the result of search for a source of copper Kα radiation capable of yielding high intensity (not less than 300 roentgens per minute at 30 cm. from the target) for periods of many hours at a time. Experience with hot cathode tubes invariably indicated gradual loss of intensity of the copper radiation due to deposition of filament material upon the anode. This objectionable feature led to experiments with gas-type tubes.

The gas tube finally adopted employs essential features described by Wyckoff and Lagsdin[1]. The design, however, has been reworked for the purpose of better adaptation to the particular problem in hand. Simplification of machine work and utilization of standard parts have also received attention.

The drawing (figure 1) shows the tube in longitudinal section. With a few exceptions, where other materials are specifically mentioned, metal parts are machined from rolled brass. Cast brass might be used for the clamping rings but danger of leaks precludes its use for other parts.

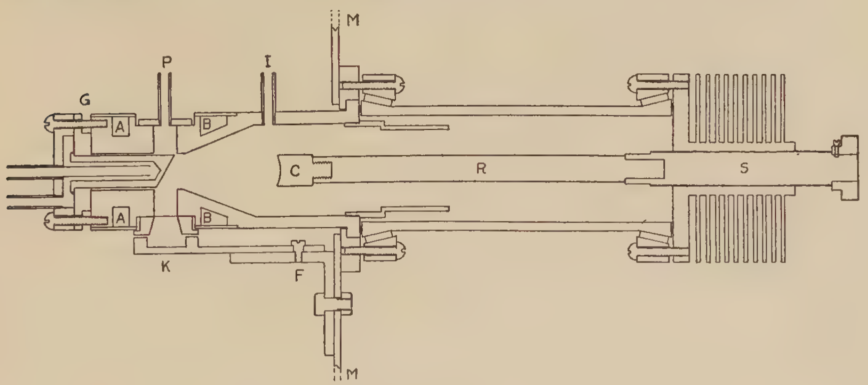


Fig. 1. Longitudinal section of x-ray tube.

The aluminum cathode tip C screws into the copper cathode rod R which in turn is soldered into the cathode screw S. This screw, which has a fine thread (40 per inch), fits closely in the cathode radiator. The radiator is turned from a solid piece of brass, using a parting tool for cutting the spaces between the cooling fins. A mixture of grease and powdered graphite is used on the cathode screw. The graphite prevents the grease from flowing and affords one of the best safeguards against possible sticking of the closely fitted screw. It tends also to improve

[1] R. W. G. Wyckoff and J. B. Lagsdin, R. S. I. 7, 35 (1936).

thermal contact between the screw and the radiator. Cooling of the radiator may be improved by directing a blast of air on the cooling fins.

The glass insulator between the radiator and the body of the tube is a six-inch length (a length which serves to establish the scale of the drawing) of two-inch diameter Pyrex pipe. Before assembling, both ends of the glass pipe are ground flat to remove the small grooves moulded into them. Care is also taken to see that the ground ends are perpendicular to the axis of the tube so that the cathode will be properly centered. This condition can best be checked by observing the cathode through the anode end of the tube before the anode assembly is put in place.

Brass clamping rings drilled to accomodate eight 10-32 machine screws hold the metal-to-glass joints together. With respect to inside diameter and taper, dimensions of these rings are taken from the iron joint flanges supplied by the Corning Glass Works. The regulation inserts may thus be used as a filling and cushioning material between the rings and the glass.

Air tight seals at the ends of the glass tube are obtained by using 0.040-inch (five ampere) fuse wire gaskets. These ring-shaped gaskets are made by fusing together the ends of the wire in a very small gas flame. If both glass and metal surfaces have a smooth finish, rather moderate pressure on the gaskets will prevent leakage.

About one-half the length of the body of the x-ray tube, beginning at the anode end, is turned down to fit inside the piece of one-sixteenth-inch wall tube which closes the periphery of the annular water jackets A and B. This tube is sweat soldered in place before any machine work is done at the window openings. The two annular water jackets are connected by four holes passing through points forty-five degrees from the window openings. Circulation is in accordance with the schematic diagram shown in figure 2. Radial passages in this diagram represent holes parallel to the axis in the actual tube in which the annual passages A and B are of the same diameter. Barriers at points ninety degrees from both inlet and outlet assure circulation of water through jacket B. The inlet and outlet tubulations extend through oversize holes drilled through the outer edge of the anode assembly.

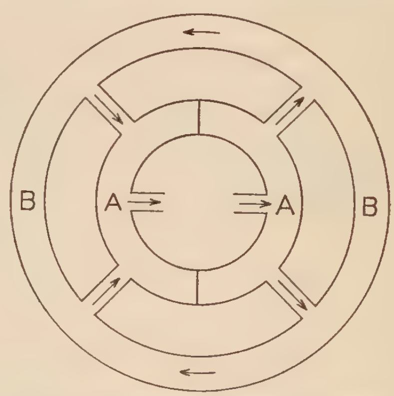


Fig. 2. Schematic diagram of water circulation. In the actual tube the annual water jackets A and B are of the same diameter and all connecting passages are parallel to the axis of the tube. The four connecting passages between A and B are drilled through the brass between the window openings.

A tubular shield which screws into the cathode end of the tube body intercepts much of the sputtered meal which would otherwise be deposited upon the inside surface of the glass.

The anode assembly consists of two circular plates plus the anode proper which is soldered into the central opening of the inner plate. Part of the edge of this plate is turned away to form the groove G. This groove exposes the junction between the anode assembly and the tube body in such manner as to permit external sealing without danger of air leakage

around the clamping screws. Water leakage from the anode jacket is prevented by a cemented paper gasket between the plates.

Water from the tap is passed directly into the central tube of the anode assembly so that maximum cooling effect is obtained just behind the focal spot. Then, by means of a short U-shaped rubbed connection (with metal or glass insert to prevent kinking) the same water is passed through the cooling passages of the body of the tube.

Four window openings are provided but when an oblique target is used three of these openings are covered by one-eighth-inch brass plates. Surfaces milled flat around the openings facilitate mounting these plates and other window fittings. All these fittings are made interchangeable and are fastened with four filluster head screws well countersunk to permit sealing over the heads. In the particular set-up shown in the drawing the pumping connection P is screwed and soldered into one of the plates covering an unused window opening.

For copper Kα radiation a nickel window, which also serves as a filter, is used. A thickness of 0.025 mm., which transmits approximately 35 per cent of the desired radiation, provides sufficient filtering for most purposes. The window is permanently fastened to its mounting ring by sweating it in place with solder, taking care to see that no solder flows onto the part which is to transmit the radiation.

After the tube is completely assembled the points around the window openings and at the junction of the anode assembly and body are sealed with beeswax. Application of this sealing material is best accomplished by placing the tube well above a flame until it is heated to a temperature somewhat above the melting point of the wax. The beeswax, which has been melted, is then applied with a small water color brush. Any surplus may be wiped off with a cloth, taking care not to remove the slight fillet at the junctions to be sealed. This fillet should be apparent at all junctions after the wax has hardened. Cooling may be accomplished quickly by passing water through the water jackets.

The tube is pumped continuously by a good fore pump while correct pressure for operation (about 0.02 mm. of Hg) is maintained by adjusting the flow of air into inlet I. The line to the pump should be made of metal or glass tubing and if it is necessary that it be several feet long the diameter should be increased accordingly. The ends may be drawn down to fit the usual rubber connections, but these connections should be made as short as possible.

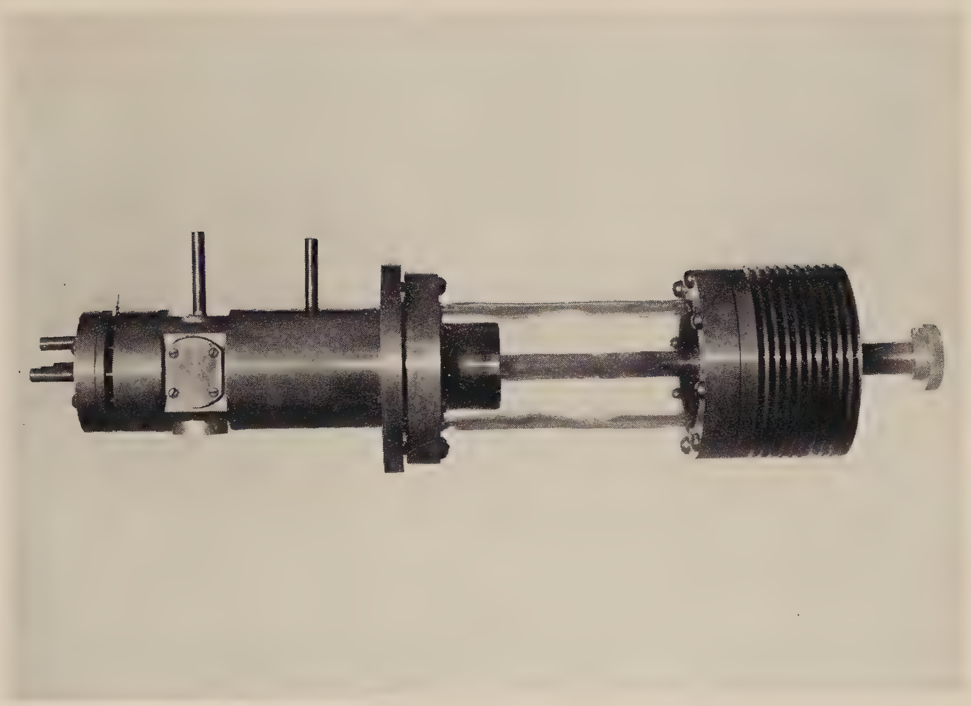
In view of the fact that the tube does not immediately attain a steady state when the voltage is applied, a shutter is provided for starting and stopping the exposures. It consists of a shallow cup K mounted on an arm which pivots about the screw F in the mounting bracket. The shutter is pulled open by a string and is closed by a spring. Adjustable stops are provided for both open and closed positions. The shape of the shutter cup and window ring is such that radiation must be scattered at least twice before it can escape through the clearance space between the cup and ring. In practice this clearance is made as small as possible by bringing the parts into contact. Tests made by prolonged exposure of dental films at various points within a few centimeters of the shutter fail to show any indication of leakage. With the clearance increased to 0.2 mm. leakage is appreciable but is confined very closely to the plane of the clearance space.

Both tube and shutter are mounted on a one-eighth-inch steel panel M M. The tube flange is fastened to the panel by three screws not shown in the drawing. A radial slot, also not shown, permits removal and replacement of the tube without interfering with the tubulations P and I.

The writer desires to express his appreciation of the fact that construction of the tube was made possible by Prof. John W. Gowen of the Genetics Department of Iowa State College.

PLATE I
X-Ray Tube

PLATE I



OCCURRENCE OF ACROLEIN AS AN INTERMEDIATE DURING THE FERMENTATION OF GLYCEROL BY THE COLI-AEROGENES BACTERIA

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The use of fixing agents designed to remove intermediate products of dissimilation from further attack (Abfangverfahren of Neuberg) has aided materially in the development of our present concepts of bacterial metabolism. In the present investigation, the fixation technique has been employed with the coli-aerogenes bacteria for further elucidating the intermediary dissimilation of glycerol. Acrolein has been isolated and identified as a probable intermediate in the dissimilation of glycerol by *Citrobacter freundii.*

EXPERIMENTAL

The organisms were grown in a medium of 20 gm. glycerol, 2 gm. ammonium sulfate, 100 ml. 0.5 M phosphate buffer (pH 7.7) and water to make 1 liter. Dimethyldihydroresorcinol (dimedon) was added as the fixing agent to a concentration of 0.33 per cent. It was neutralized to phenolphthalein with sodium hydroxide, sterilized separately in 200 ml. water and added aseptically to the medium at the time of inoculation. Incubation took place at 30° C. for 8 days. The cultures of Escherichia were obtained from the American Type Culture collection; cultures of Aerobacter were those described by Burkey (1928). *Citrobacter freundii* (no. 147) was a strain isolated in this laboratory.

Citrobacter freundii grew well in the medium, developing a crystalline deposit on the sides of the flask after 3 days. In the same medium one culture each of *E. coli* (ATC26) and *Aerobacter indologenes* developed sparse growth and failed to produce the crystalline deposit. Incubation was discontinued after 8 days. The crystalline material was removed by filtration, washed and crystallized three times from ethyl alcohol, dried over H_2SO_4 and weighed, giving a yield of 2.06 gm. It was found to melt at 215° to 216° C. (corr.). A test for phosphorus was negative. The dimedon compound was soluble in dilute potassium hydroxide, but insoluble in 33 per cent potassium hydroxide. Acidification of the alkaline solution precipitated the compound. Although soluble in cold sulfuric acid, it was precipitated by dilution with cold water. The compound was insoluble in ethyl acetate, isoamyl alcohol, acetone, benzene, and 2 N hydrochloric acid.

A study of the literature revealed no dimedon derivative of any compound with such a melting point which could be considered as a likely product of this fermentation. Analysis of the derivative gave C, 71.52; 71.53 and H, 7.99; 8.09. A Rast molecular weight determination gave 323. The calculated values of carbon and hydrogen for an empirical formula $C_{19}H_{26}O_4$ having a molecular weight of 318.2 are C, 71.65 per cent and H, 8.23 per cent. A list of possible compounds having a molecular weight and

empirical formula in relative agreement with observed values included the dimedon derivative of propionaldehyde and acrolein. The melting points as given in the literature for the dimedon derivatives of propionaldehyde were found to be in very good agreement. Klein and Linser (1929) and also Kao and Yen (1932) published data which gave 155° C. as the melting point of the propionaldehyde. Klein and Linser, however, gave a melting point of 135° C. for the acrolein derivative while Vorländer (1929) listed the melting point as 192° C. after softening at 186° C.

It was necessary to prepare a pure sample of the dimedon derivative of acrolein because of these very obvious discrepancies in the literature. Ten grams of acrolein (Eastman) were dissolved in 290 cc. of water and 30 cc. of this solution added to 50 cc. of water containing 500 mg. dimedon. After standing 3 days at room temperature, the material which separated was filtered and dissolved in alcohol. The material which crystallized was filtered and washed with dry ether. After it was air-dried, the melting point was found to be 215° to 216° C. When the derivative isolated from the fermentation medium was mixed with the prepared derivative, there was no depression in the melting point (215° to 216° C. [corr.]).

In subsequent tests, duplicate tubes of the glycerol-dimedon medium were inoculated with one loop each of 24-hour broth cultures of *Escherichia coli* (nos. 26, 4163 and 4265), *Citrobacter freundii* (nos. 147, 176, M8BK) and *Aerobacter indologenes* (no. 23), *A. decolorans* (no. 24), and *A. salicinovorum.*

After incubation for 7 days, all tubes of Citrobacter showed crystalline deposits, whereas those of *E. coli* were negative. Tubes inoculated with *A. indologenes* and *A. salicinovorum* were negative; whereas those with *A. decolorans* showed a crystalline deposit. The latter may have been an acetaldehyde derivative.

FERMENTATION OF GLYCEROL BY CITROBACTER FREUNDII IN THE PRESENCE OF CALCIUM SULPHITE

Citrobacter freundii was incubated for 8 days at 30° C., in a medium of 2 per cent glycerol, 0.2 per cent $(NH_4)_2SO_4$, 0.1 per cent K_2HPO_4, 1.0 per cent $CaCO_3$, and 1.0 per cent $CaSO_3$. At the end of this period, a 200 ml. portion of the liquor was filtered and treated with an equal volume of a saturated solution of 2,4-dinitrophenylhydrazine hydrochloride in 2 N HCl. A copious yellow precipitate which separated immediately, was removed by filtration after standing in the refrigerator for 12 hours.

Treatment of the precipitate with 10 per cent sodium carbonate solution gave a dark brown solution and an insoluble orange residue. Acidification of the sodium carbonate solution yielded a yellow precipitate which was purified by redissolving and precipitating in 10 per cent sodium carbonate with final crystallization from alcohol. The dried compound melted at 212° to 213° C. (uncor.) and there was no depression of the melting point when mixed with an authentic preparation of the 2,4-dinitrophenylhydrazone of pyruvic acid.

The sodium carbonate insoluble residue was dissolved in alcohol, crystallized and dried. The melting point of the dried preparation was 165° to 166° C. and showed no depression when mixed with an authentic preparation of the 2,4-dinitrophenylhydrazone of acetaldehyde.

Another portion of the fermented liquor was distilled after the addition of sodium carbonate to retain pyruvic acid. The distillate yielded a white crystalline precipitate with dimethyldihydroresorcinol which melted at 136° C. (uncor.) and retained the same melting point after mixing with a known dimedon derivative of acetaldehyde.

Fermentation in the presence of sulfite led to the isolation and identification of pyruvic acid and acetaldehyde as fixation products in the dissimilation of glycerol. No other aldehydic or ketonic compound precipitable by 2,4-dinitrophenylhydrazine could have been present in significant quantities. Thus the presence of calcium sulfite led to the fixation of pyruvic acid and acetaldehyde while acrylic aldehyde was fixed in the presence of dimedon.

SUMMARY

In a medium containing only inorganic salts, glycerol and dimedon, a culture of *Citrobacter freundii* yielded a relatively large quantity of a water insoluble crystalline material. The compound has been identified as the acrylic aldehyde derivative of dimedon (2,2-[Acrylidene] bis [5,5-dimethyl-1,3-cylcohexanedione]).

When tubes of the same medium were inoculated with representative strains, three cultures of Escherichia gave no deposit, four of Citrobacter gave the characteristic crystalline precipitate. One of three Aerobacter cultures gave a precipitate. In a similar medium with calcium sulfite as the fixing agent, fermentation by the *Citrobacter freundii* culture resulted in the accumulation of pyruvic acid and acetaldehyde.

The identification of acrolein as a possible intermediary in the dissimilation of glycerol should be of value in determining the mechanism of its breakdown.

REFERENCES

BURKEY
1928. Iowa State Coll. Jour. Sci. 3:57-100.

KAO and YEN
1932. Science Reports Natl. Tsing Hua Univ. Ser. a, 1:185-188.

KLEIN and LINSER
1929. Mikrochem. Pregl Festschr., 204-234.

VORLÄNDER
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THE EFFECT OF THE CONCENTRATION OF MANNITOL UPON THE PRODUCTION OF LEVULOSE BY THE ACTION OF ACETOBACTER SUBOXYDANS[1]

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In a previous communication from these laboratories, Fulmer, Dunning, Guymon, and Underkofler (1936) presented data on the effect of the concentration of sorbitol upon the production of sorbose by the action of *Acetobacter suboxydans*. The rate of formation of sorbose was decreased by increasing concentrations of the substrate. However, the final yield of sorbose was practically independent of the concentration of the sorbitol up to and including 35.0 per cent; there was a marked drop in yield at 40.0 per cent. At the former value, 35 per cent, the concentration of sorbose reached the high value of 28 grams per 100 ml. of medium. The use of high concentrations of the sorbitol affords an easy method for the laboratory production of sorbose with the handling of minimum volumes of liquids. Wells, Stubbs, Lockwood, and Roe (1937) have adapted the process to the use of submerged growth of *Acetobacter suboxydans* in a rotating drum developed for the production of gluconic acid by molds.

Studies have also been made in these laboratories on the effect of concentrations of other polyhydric alcohols upon the yields of the corresponding ketose sugars through the action of *Acetobacter suboxydans*. Underkofler and Fulmer (1937) found that maximum yields of dihydroxyacetone were not obtained in concentrations of glycerol above 6.0 per cent. According to Whistler and Underkofler (1938) the concentration of meso-erythritol should not exceed 4.5 per cent for maximum production of *l*-erythrulose. Dunning, Fulmer, Guymon, and Underkofler (1938) investigated the action ot *Acetobacter suboxydans* upon *i*-inositol. The organism could not be subcultured beyond the fifth transfer upon an inositol-yeast extract medium. However, the addition of very small amounts of sorbitol permitted indefinite subculture and vigorous oxidation to a diketo-inositol. Data, to be published later, show that low concentrations of erythritol, glycerol, mannitol, and dextrose can replace sorbitol in the above medium.

The results mentioned above show a variation in the relation of concentration of substrate and yield of the corresponding ketose sugar. The maximum concentrations permissible without interfering with the final yield are 35 per cent for sorbitol, 6 per cent for glycerol, and 4.5 per cent for meso- erythritol; in the case of *i*-inositol an additional substrate, in low concentration, is necessary for continuous subculturing and oxidation of the substrate.

While mannitol, a steroisomer of sorbitol, has been reported by earlier workers to be oxidized by *Acetobacter suboxydans*, no systematic studies

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have been reported on the concentration-yield relationships. The present communication deals with the development of conditions giving high yields of levulose. The culture employed is the same as that used in previous studies in these laboratories. The stock cultures were carried on yeast extract-glycerol-agar slants. The cultures used for inoculation were kept active by transfer, each 48 hours, into a medium containing 0.5 g. of yeast extract (Difco powdered) and 5 g. of mannitol per 100 ml. of medium in 300-ml. Erlenmeyer flasks. Since disturbing an active surface growth of the culture materially reduces the yield of the fermentation product, each datum presented in the following tables represents the analysis of the contents of a separate flask. The levulose determinations were made by the Shaffer-Hartmann (1920) method; the dextrose factor was multiplied by 1.1 to obtain the levulose value. Crystalline levulose was prepared from the fermented medium by a procedure similar to that employed by McGlumphy, Eichinger, Hixon, and Buchanan (1931) for the separation of the sugar from the juice of the Jerusalem artichoke.

In table 1 are presented data showing the effect of varying surface-volume ratios (sq. cm. area per 1 ml. volume) upon the production of levulose. While the rate of reaction is slower with decreasing ratio, the

TABLE 1. *The effect of surface-volume ratio (sq. cm. area per 1 ml. medium) upon the production of levulose*

Time days	Surface-volume ratio					
	2.36	1.195	0.895	0.589	0.345	0.200
3	96.0	82.0	54.0	39.0	24.0	14.3
5	95.0	94.0	92.0	83.0	41.3	43.3
7	95.0	94.0	94.0	92.0	82.0	62.0
9	.....	.....	.....	.....	92.0	81.0
14	.....	.....	.....	.....	.....	93.0

final yield is little influenced at ratios varying from 2.36 to 0.200. Table 2 shows the relation of yield of levulose to the concentration of mannitol. Concentrations of the substrate up to and including 25 per cent slow down the rate of reaction, but the final yield is unchanged; at a concentration of 30 per cent both the rate and final yield are decreased.

TABLE 2. *Effect of concentration of mannitol upon production of levulose* (surface-volume ratio = 1.195)

Time days	Concentration of mannitol				
	10	15	20	25	30
3	82.8	72.0	42.0	13.6	1.0
5	93.5	95.0	92.0	66.0	15.0
7	94.0	95.0	93.0	93.0	49.0
9	....	....	93.0	94.0	69.0
14	....	....	....	....	80.0

SUMMARY

Data have been presented on the effect of surface-volume ratio and concentration of mannitol upon the production of levulose by the action of *Acetobacter suboxydans*. Decreasing surface-volume ratio decreases the rate of reaction but has little effect upon the final yield through the range studied. Up to and including 25 per cent of mannitol the rate of reaction is decreased while the final yield of levulose is little affected; higher concentrations decrease both the rate and the final yield.

That is, the concentration-yield relationships for the transformation of mannitol to levulose are similar to those for the oxidation of sorbitol to sorbose and are dissimilar to those involving the oxidation of glycerol, meso-erythritol, and *i*-inositol.

LITERATURE CITED

1. Dunning, J. W., E. I. Fulmer, J. F. Guymon, and L. A. Underkofler
   1938. Science, 87, 72.

2. Fulmer, E. I., J. W. Dunning, J. F. Guymon, and L. A. Underkofler
   1936. J. Am. Chem. Soc., 58, 1012.

3. McGlumphy, J. H., J. W. Eichinger, R. M. Hixon, and J. H. Buchanan
   1931. Ind. Eng. Chem., 23, 1202.

4. Shaffer, P. A., and A. F. Hartmann
   1920. J. Biol. Chem., 45, 365.

5. Underkofler, L. A., and E. I. Fulmer
   1937. J. Am. Chem. Soc., 59, 30.

6. Wells, P. A., J. J. Stubbs, L. B. Lockwood, and E. J. Roe
   1937. Ind. Eng. Chem., 29, 1385.

7. Whistler, R. L., and L. A. Underkofler
   1938. J. Am. Chem. Soc., 60, 2507.

EQUIPMENT FOR RADIOGRAPHY WITH SOFT X-RAYS

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Radiography, as applied to the study of small objects (for example, insects), requires the use of soft x-rays in conjunction with fine grained photographic plates from which suitable enlargements may be made.

Goby (2), Friche (1), Sherwood (4), and others have developed the soft x-ray technique for studying small biological specimens, but this application has not become general. The slow development of this field can doubtless be attributed to the lack of information concerning the apparatus required, and also to the high cost of such equipment. The purpose of this paper is to present the important features and methods of constructing such equipment.

Sherwood (4), following the original work of Goby (2), has successfully applied the soft x-ray technique to stereoscopic and motion picture work. As in ordinary radiography, the object to be rayed must be close to the photographic plate so that a shadow image may be formed by radiation from the x-ray tube. The plate must be protected in a holder, opaque to ordinary light, but transparent to x-rays. Sherwood recommends, because of its lack of texture, an infra-red gelatin filter (Wratten No. 87) as a protecting window in the holder. Thin black paper can be used, although its structure may appear in the radiograph. With the average specimen, however, this is of little consequence, and since paper is not so easily damaged by moisture, scratches, or fingerprints, it is often preferable.

Sherwood found that a variety of photographic materials were suitable, but recommends slow lantern slide plates as the most satisfactory. Enlargements of from five to ten diameters retaining the details of the image are attainable with such plates.

The x-ray wave lengths used in this work included the range between 3.8 and 0.82 Ångströms. This represents tube voltages of 4 to 15 Kv. peak. The lower voltages must be used for very thin specimens in order to preserve contrast.

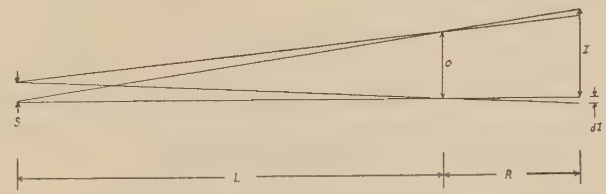


Fig. 1

As in other types of photography, the sharpness of the negative image determines the magnification possible in the final print. With x-rays, the sharpness depends on the diameter of the focal spot, the dimensions of which are of primary importance in the tube design.

Figure 1 reprsents x-rays originating from a source of width S, passing through a structure element of width O, and forming an image of width I. Let the distance between the source or focal spot and the object be L and that between the object and the image be R. Then clearly,

$$I = \frac{O(R + L)}{L}$$

$$\text{and}\ \ dI = \frac{RS}{L}$$

As a consequence of these two equations we have the following stipulations:

1. For I to reproduce faithfully, O, R must be small.
2. For dI to be small, R and S must be small or L large.
3. dI is independent of I.

Since dI is the factor which controls diffusion in the image, we may set its limiting magnitude and determine the other constants to give this magnitude. If 1.5 minutes of arc is assumed for the limiting angle of resolution of the eye, the size of the least resolvable object when viewed at a distance of 25 cm. (limit of distinct vision) is approximately 0.1 mm. If the radiograph is to be enlarged five times, a value of 0.02 mm. is obtained for dI. If L is 250 mm. and R is 5 mm., S is found to be 1.0 mm. Thus a 1.0 mm. focal spot would provide sufficient sharpness. For thin speci-

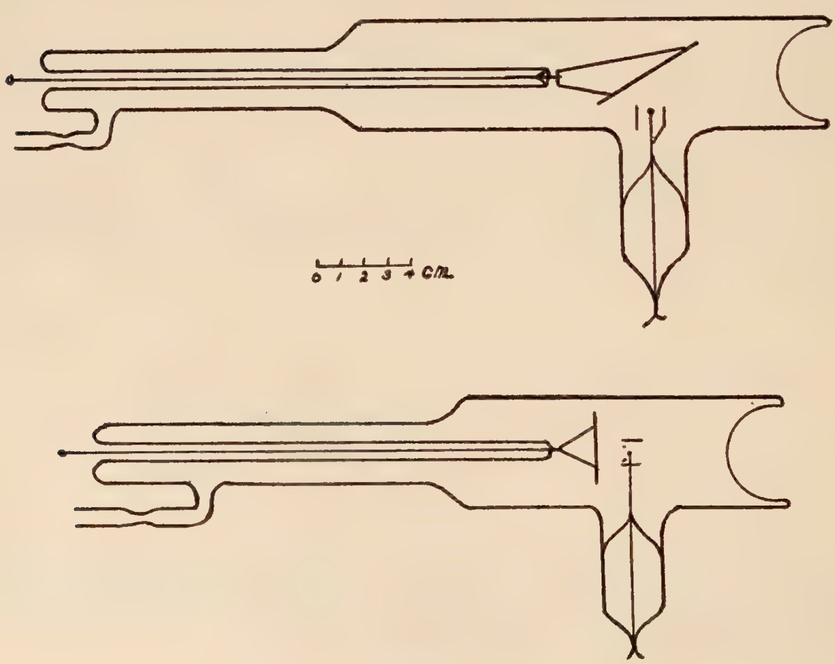


Fig. 2

mens, however, R could be reduced by half, which would allow a 2 mm. focal spot.

Another important design feature is the permissible thickness of the pyrex window. Estimations made from transmission tables for aluminum (Kirchner (3)), indicate that the window thickness should be less than 0.05 mm. for satisfactory results in the long wave length region.

TUBE CONSTRUCTION

Two types of tubes, illustrated in figure 2 were evolved. In the ninety-degree type, the x-rays were emitted from an oblique face of the anode, along the axis of the tube, while in the ring cathode design, the x-rays were projected back through the cylindrical shield. Both tubes utilized similar construction features.

The anode and cathode focusing cylinder were made of 0.15 mm. sheet molybdenum. A single loop of 4 mil tungsten wire, 4 mm. in diameter, was located coaxially within the cylinder, and mounted about 1 mm. below the edge of the shield. Filament leads and anode seal were of 20 mil tungsten. The inter-electrode distance was 10 mm.

Thin pyrex windows have been described by Slack (5). The general scheme of construction is, first, to seal off the end of a glass tube of the proper diameter; then after thinning the glass by drawing and working, the end is sucked in to form a hemisphere. As a result of this shape, the atmospheric pressure is distributed along the glass wall as a tension, after evacuation, and enables the wall to withstand the pressure inspite of its thinness.

The principal steps in the construction are given in figure 3. The operations, on the whole, are routine glassblowing procedures. To form the thin window, a gentle flame of high oxygen precentage compared to air is recommended.

TUBE EXHAUSTION

The production of an x-ray vacuum, although now a common process, involves careful procedure and scrupulous attention to details. The degree of vacuum required by a Coolidge tube amounts to a pressure of the order of 10^{-4} to 10^{-5} mm. of mercury. With modern pumping systems such pressures are easily obtained, but the success of the exhaustion process depends primarily on the removal of the occluded gas from the metal parts. It was found that with the small metal parts used, the out-gassing process could be most easily accomplished by means of electron bombardment.

ELECTRICAL EQUIPMENT

A diagram of the electrical circuit is shown in figure 4. The 500 VA transformer T_1 has a 15,000 volt secondary, the primary voltage being 115 volts. One end of the secondary was grounded. The current was fed in through an auto transformer T_3 with the voltmeter V_1 attached to read input voltages. T_2 was a radio tube filament transformer with a 5-volt winding. The 6-ohm variable resistance R in its secondary circuit varied the filament current. The proper filament current could be deter-

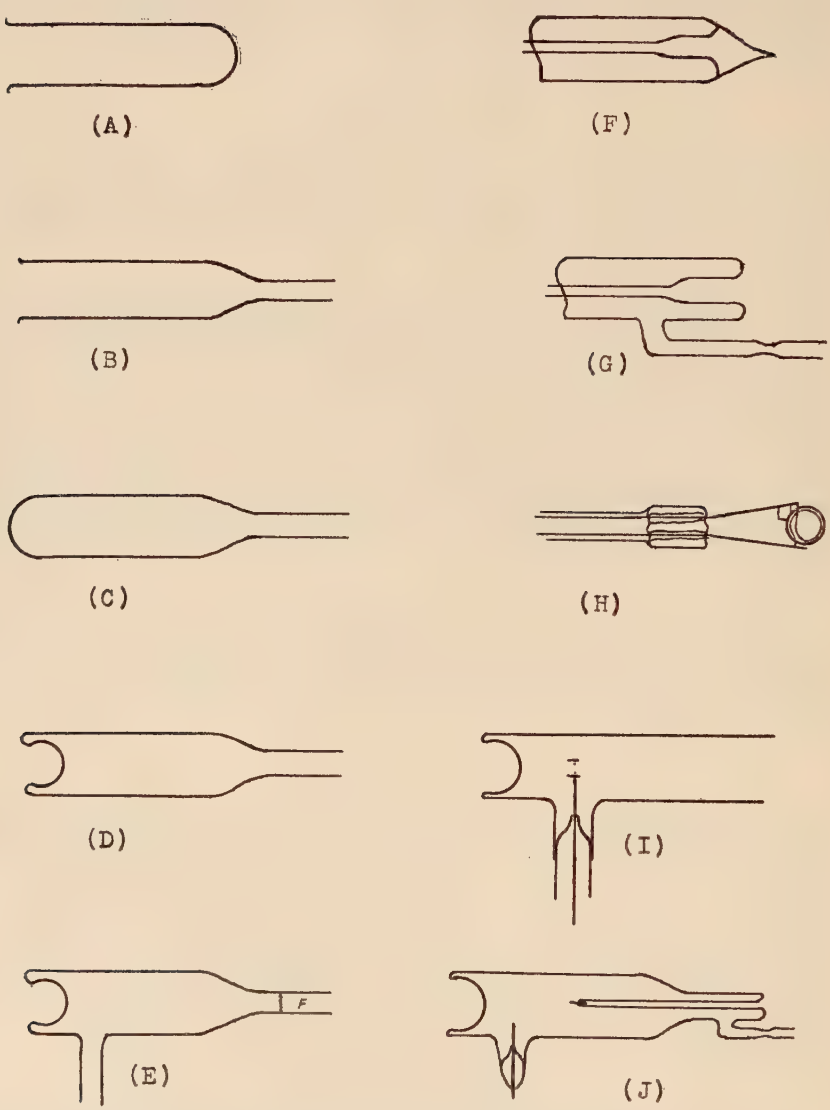


FIG. 3. A, Test tube; B, Shoulder seal; C, Thinned end in preparation for window; D, Window formed; E, Side tube attached; F, Enlarged section of neck with re-entrant tube; G, Exhaust tube attached; H, Cathode assembly; I, Cathode assembly seal; J, Finished tube.

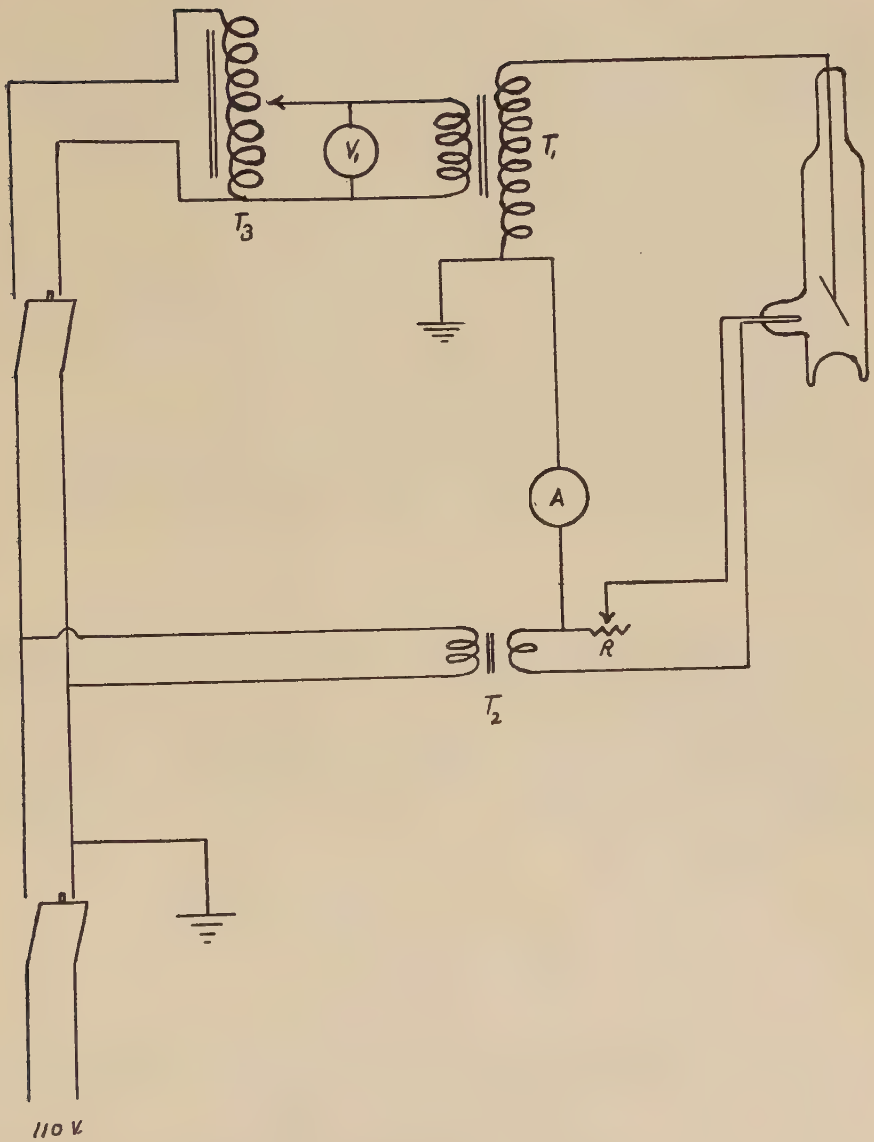


FIG. 4

mined best, however, by observing the tube current read on the milliameter A inserted in the ground side of the system.

TUBE MOUNTING AND SPECIMEN HOLDER

When viewing an object from the distance of most distinct vision (25 cm.) the eyes subtend an angle of approximately fifteen degrees. Thus, stereoscopically, the eyes receive two views of an object, at 25 cm., from positions fifteen degrees apart. If, then, a stereoscopic radiograph is to be made, the image forming rays should originate from sources which are located at the positions occupied by the eyes when focused on the object.

Only one source of x-rays is necessary, for it may be shifted from one position to another, and the two views recorded separately. Provision must be made, however, for changing the photographic plate between the exposures.

The supporting rack for the tube was designed to accomplish the stereoscopic motion, figure 5. The tube itself was clamped to a section of insulating board which could be moved vertically in the rack. This feature allowed adjustment of the anode-film distance.

Sherwood (4) mentions several types of specimen platforms. A simple one was constructed from an old lantern slide carton. It is included in Plate I, upper figure. An opening was cut in the top of the cover, and a thin sheet of black paper pasted in to form an opaque window. The sensitive plate was then held against the window by the spring action of cotton wadding.

RESULTS

Windows of 52 microns thickness were successfully fabricated. These transmitted sufficient radiation for wave length below 1.3 Ångströms. Pinhole pictures of the focal spot indicated that the radiating area was limited to a diameter of 2 mm.

The radiated energy from the tube was meaured with an "Open Air Ionization Chamber" (6) at several tube voltages and at an anode-ionization chamber distance of 9.4 cm. The values obtained are shown in the table below.

Kilovolts peak	Intensity in R units
22.6	51.7 R per minute
20.3	37.2
18.1	23.4
15.7	12.9
13.5	6.3

The tube could be operated continuously at a voltage of 15,000 R.M.S. and a current of 2 milliamperes, which represents a heat dissipation of 30 watts.

Plate I, lower figure is a stereoscopic view of a cockroach, taken with the apparatus, and presents a sample radiograph of a typical soft x-ray specimen.

In conclusion we wish to express our thanks to Mr. L. E. Pinney for his many helpful suggestions.

LITERATURE CITED

1. Fricke, Hugo
1932. Insect Radiography. Jour Biol. Phot. Assoc., **1**: 90.

2. Goby, Pierre
1913. Une Application Nouvelle des Rayons X: la Microradiographie. Comptes Rendus. Acad. Sci., **156**: 686.

3. Kirchner, Fritz
1930. Allgemeine Physik der Röntgenstrahlen. Handbuch der Experimentalphysik., pt. 1, **24**: 14. Akademische Verlagsgesellschaft M. B. H., Leipzig.

4. Sherwood, H. F.
1937. The Radiography of Small Biological Specimens. Jour. Biol. Phot. Assoc., **5**: 138.

5. Slack, C. M.
1929. Lenard Ray Tube with Glass Window. Jour. Opt. Soc. Am., **18**: 123.

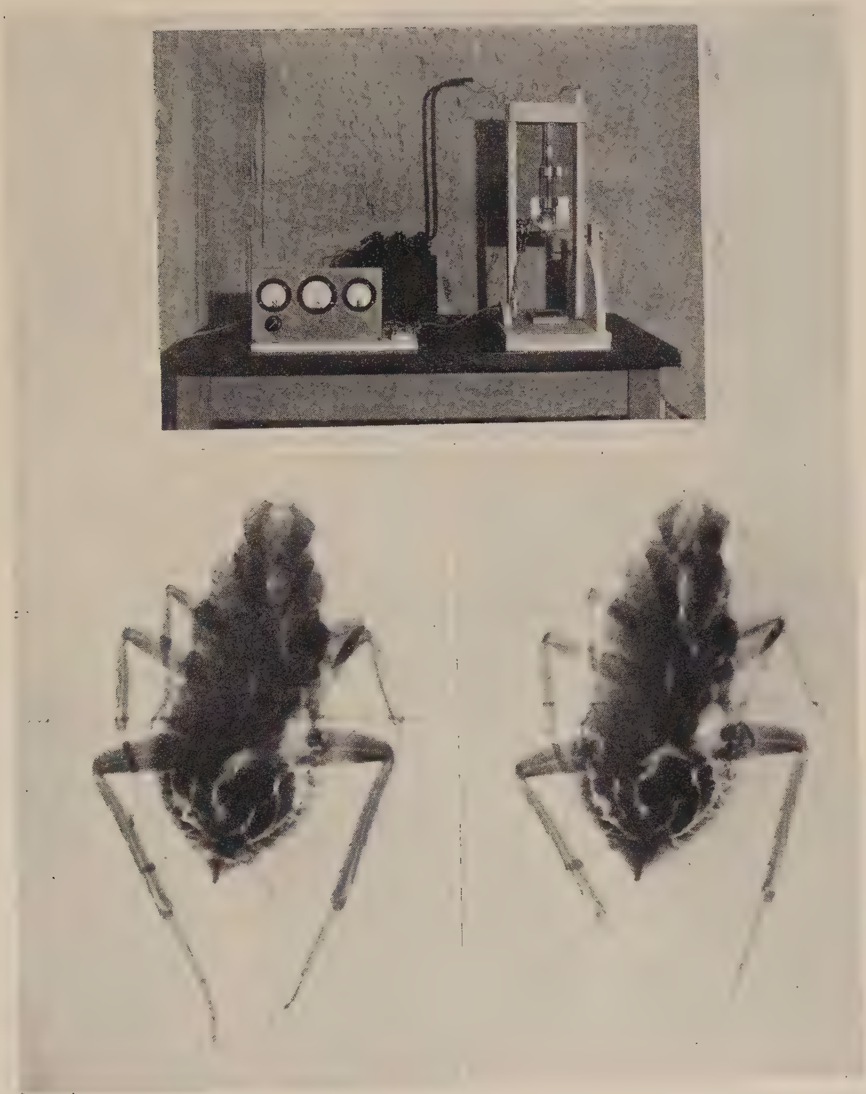
6. Terrill, H. M., and C. T. Ulrey
1930. X-Ray Technology. p. 138. D. Van Nostrand Co., New York.

PLATE I

UPPER: Stereo x-ray apparatus.

LOWER: Stereoradiograph of cockroach (*Periplaneta americana*).

PLATE I



SOME EXPERIMENTS WITH FERTILIZERS FOR EVERGREEN SEEDLINGS[1]

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From the Forestry Department, Iowa State College

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In this paper it is the purpose to record certain results of tests made with fertilizers of coniferous nursery stock carried on by advanced students in forestry at Iowa State College.

In order to meet the requirements for an increased output of small nursery grown trees now demanded in our expanded forest planting programs and to secure rapid growth and well developed stock it has become necessary to study how to maintain and increase the fertility of the soil in the nursery where the trees are produced. In outlining the work for the various students it was the design principally to test the effects of various organic and inorganic fertilizers containing nitrogen, phosphorus and potassium, and to observe the effects of acid or basic forming fertilizers on certain soils.

The inorganic nitrogen fertilizers come in two distinct forms: (6) those which have the more readily soluble and leachable nitrate form and those which have the ammonia compounds. The organic nitrogen may be obtained from materials such as dried blood and bonemeal. The steaming of the bonemeal will remove the less soluble fats and proteins. The phosphorus is available in three main groups: the ammo-phosphates and superphosphates in the first group; the reverted or citric-soluble phosphoric in the second; and thirdly the phosphatic residues which are also citric soluble. In still another group is the phosphate rock. Potassium fertilizers occur in the form of potassium chloride, called muriate of potash, potassium sulfate, potash magnesia, potassium nitrate and hardwood ashes, etc. These are generally quite soluble and readily available, but the first two may be acid forming. Phosphorus compounds discussed by Pierre (10) are not strongly acid or basic forming in the soil, in fact, the purer forms tend to neutralize it but certain combinations may be used such as tricalcium phosphates, bi-, or monocalcium phosphates which reduce acidity. Petersen (9) states that sodium phosphate has the same effect.

The conditions which may influence the results, aside from the fertilizers themselves, are the species of trees used in the tests. It seems that the same reagents bring about different results for pine, spruce, and Douglas fir, and that the white and yellow pines react differently to the same treatment. Again the planting stock may respond in different ways

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[1] These studies have been under the direction of the senior author. The theses and reports forming the bases for this article were prepared by Lester Marriage, Irving Christensen, Joseph Stoeckler, B. A. Bateman, A. L. McComb, Gordon Gray, and C. E. Anderson. It was a part of Mr. Stump's work to collect the data from various theses and it remained for Professor Larsen to select the more suitable material and prepare the report.

Professor B. J. Firkins in the Department of Agronomy has given valuable assistance in suggesting the kinds of fertilizers, quantities and combinations to use.

according to age, for those of the second year in the seedbed ordinarily show more definite trends than those of the first year.

No doubt one of the chief causes of variations lies in the soil or the media used for the tests, the physical and chemical constitution of which are capable of a wide range of variations, and the soil itself at the various nurseries where the tests have been made account in no small degree for the variable responses reported by the investigators in this field. Wahlenburg (11), experimenting on what is a distinctly acid soil in Montana, obtained best results with lawn dressing, blood and bonemeal, ammonium sulfate, dried blood, potassium nitrate, ammonium and calcium nitrate. Lunt (4), who used bloodmeal, hyper-humus, magnesium sulfate, milorganite, peat moss, red pine duff, sulfur tankage, and Nitrophoska on acid soils in Connecticut, obtained poor response with acidifying reagents and better responses with Nitrophoska, tankage, potassium singly or with phosphorus.

The first tests in these series were made at the older nursery near the Armory. The land here is a part of the general upland plain but with here and there a minor depression which has caused the soil to become heavy and slightly alkaline. At the State Avenue nursery southwest of Ames, the land is largely upland plain with more pronounced slopes. The soil is, therefore, better drained, less compacted, and from neutral to slightly acid. Anderson's work was carried on on the upland section of this plain and the experiments of McComb and Gray on the gentle easterly slopes. Christensen and Stoeckler ran their tests in the college greenhouse.

Marriage (5) conducted his experiments at the Armory nursery in 1931. The soil is a Wisconsin drift and the propagation beds are located in a section with rather heavy and poorly drained soil. Some rotted manure had been applied to all of the seed beds at time of installation. Six different fertilizers were used in the experiment (see table 1). Of these the aluminum sulfate was used in order to try the effect of an acid forming substance, and sodium nitrate to test the effect of a basic forming material. The seedbed units selected for treatment were 4 x 6 feet and in the middle of each a strip 2 x 4 feet was left as a check, the fertilizers being applied to the remainder, after the germination was complete and the new plants established. Water was applied immediately after the treatment in order to prevent injury to the stock by the concentrated chemicals.

At the end of the second season twenty-four specimens of the stock were selected from each treated and untreated plot. (Unfortunately the Norway spruce which was treated with sodium nitrate and ammonium sulfate were removed from the beds before stock could be selected for measurements.) Each seedling selected for records was washed thoroughly to remove all foreign material. The stock was allowed to air dry before the weights and measurements were taken. The first lateral root was used as the division point between the tops and roots. In computing the percentage of stem and of roots the controls were taken as 100. The top-root ratios expressed in table 1 is the percentage of weights in the tops to that of the roots.

Looking at the results of these first tests it will be observed that the 2-12-6 at 250 pounds per acre, the aluminum sulfate at 200 pounds per acre and the sodium nitrate at 200 pounds definitely stimulated top growth of the white pine, and that the aluminum sulfate was the only treatment which produced increase in the roots. Ammonium sulfate on the other

TABLE 1. *Effect of fertilizers on top and root growth of white pine, Douglas fir and Norway spruce*

Fertilizer	Top-root ratio percentage				Growth in percentage						Pctg. air dry weight compared to control		
	Rate per acre	White pine	Nor. spruce	Doug. fir	White pine		Douglas fir		Norway spruce		White pine	Doug. fir	Nor. spruce
					Stem	Root	Stem	Root	Stem	Root			
2-12-6	250	62	26	95	107	100	104	78	76	92	82	106	91
Control	...	57	32	71	100	100	100	100	100	100	100	100	100
Aluminum sulphate	200	54	38	70	112	119	98	100	115	92	123	120	109
Super-phosphate ..	200	60	49	89	103	101	100	82	138	116	84	95	91
Control	...	59	41	72	100	100	100	100	100	100	100	100	100
Vigoro	250	57	54	84	97	100	94	81	142	108	101	118	121
Sodium nitrate	200	58	..	71	110	93	93	99	...	...	118	107	...
Ammonium sulfate	150	59	..	79	102	86	101	93	...	...	127	113	...
Control	...	50	..	73	100	100	100	100	...	...	100	100	...

hand inhibited growth in the pine. It was expected that an acidifying agent such as the aluminum sulfate would prove of greater value on this alkaline soil than the ammonium sulfate.

Superphosphate and Vigoro were especially beneficial for the Norway spruce, both tops and roots, and the 2-12-6 produced no positive results. In case of the Douglas fir any and all of the fertilizers used proved of no value. There was, however, considerable increase in the total weights with the aluminum sulfate.

Christensen (3) began his experiments at Ames in 1931. His purpose was to study the effect of fertilizers of organic and inorganic nitrogenous composition, and phosphatic and potash elements on the development of 2-0 white pine and Norway pine seedlings (after transplanting). These tests were made in the greenhouse, using jars containing 4 gallons of Carrington sandy loam. This soil contains by weight 45.2 per cent silt, 18.5 per cent very fine sand, 18.8 per cent fine sand, 4.6 per cent coarse loam, 8.5 per cent medium sand and 4.4 per cent clay, 4,005 lbs. total nitrogen per acre, 982 lbs. phosphorus in the surface acre and a pH of 5.25.

Two series of 28 pots were used each containing 40 pounds of soil. Each series was treated with the same amount of fertilizer. White pine stock was used in one series and Norway pine in the other.

The seedlings were planted three to the jar, spaced three inches apart and planted at the same depth as they would have been set in the nursery. Soil moisture was kept near 66 per cent of the saturation and the plants received the utmost care.

Before setting the seedlings in the jars, each plant was weighed and measured for lengths of roots and tops. All soil and surface water was removed from the plant before weighing, and the measurements were taken under water to prevent exposure and drying out of any part of the roots. As is customary in nursery practice the roots were cut to a maximum length of 8 inches below the ground line before weighing. The increase in weight will therefore show the actual amount of root used in field planting and which of the fertilizers produced the best root development within this area. The application of the fertilizers and their results are given in table 2.

At the end of seven months the trees were removed from the jars and each tree was weighed and measured separately. In the 2-0 white pine the rotted manure and superphosphate gave the best results. Dried blood and a heavy application of superphosphate were next best. The rotted manure and the dried blood produced the greatest amount of top growth. Only slight effects were noted with Nitrophoska and fresh manure. A liberal application of dried blood and a light treatment with muriate of potash plus superphosphate and the Nitrophoska gave good top-root balances. It appears that the superphosphate, the muriate of potash with superphosphate and the ammonium phosphate stimulated root growth of the white pine. The sodium nitrate plus muriate of potash, the heavy application of the muriate of potash plus ammonium phosphate decreased growth very decidedly.

Results of the fertilizers upon Norway pine differed much from those obtained for the white pine. In this species the sodium nitrate and muriate of potash gave the best increases of weight. The heavy treatment with the muriate and Nitrophoska were next in rank. Dried blood gave negative

TABLE 2. *Effect of fertilizers on seedlings of white pine and Norway pine*

	White pine				Norway pine		
	Pounds per	Stem increase	Weight increase	Survival		Weight increase	Survival
Fertilizer	acre	inches	pctg.	pctg.	Fertilizer	pctg.	pctg.
Rotted manure	16,000	5.03	217.0*	50.0	Sodium nitrate muriate potash	62.7	33.3
Superphosphate (light)	200	.61	144.5	100.0	Muriate potash (heavy)	61.6	33.3
Dried blood	100	.73	112.0*	100.0	Nitrophoska	31.1	66.6
Superphosphate (heavy)	400	.89	106.1†	100.0	Superphosphate (heavy)	30.3	50.0
Sodium nitrate (light)	100	.47	77.5	100.0	Sodium nitrate (heavy)	29.4	50.0
Sodium nitrate (heavy)	200	.47	39.5	100.0	Superphosphate muriate potash	14.8	100.0
Muriate potash (light)	100	.63	35.4	100.0	Sodium nitrate (light)	5.7	100.0
Muriate potash (heavy)	200	.14	28.3	100.0	Check	.....	100.0
Ammonium phosphate	150	.21	25.3	83.3	Muriate potash (light)	− 2.1	50.0
Muriate + superphosphate	300	.65	22.6	100.0	Ammonium phosphate	−21.1	100.0
Nitrophoska	106	.60	16.3	100.0	Superphosphate (light)	−22.9	100.0
Fresh manure	16,000	1.08	10.1	66.6	Fresh manure	−29.2	50.0
Check	.....	....	.....	100.0	Dried blood	−42.4	100.0
Sodium nitrate muriate potash	200	.85	−21.2	100.0	Rotted manure	−64.8	33.3

* Due more to top development.
† Due more to root development.

results. Superphosphate, Nitrophoska and dried blood produced the best roots, and the rotted manure stimulated height growth.

Stoeckler (8) ran his experiments in the college greenhouse in 1931 using the 4-gallon jars. He also aimed to learn the effects of various organic and inorganic fertilizers in stimulating growth and development of evergreen nursery stock. The fertilizers were used singly and in combination and at different rates of application. The Carrington loam which he used gave essentially the same tests as that used by Christensen. It showed a total nitrogen of 4,294 pounds per acre and a phosphorus of 1,238 pounds; a pH of 5.75.

Series I and II consisted of 32 4-gallon jars each of 2-0 Norway pine and Black Hills spruce (sometimes called white spruce), *Picea albertiana glauca*. All applications were run in duplicate with four controls. The fertilizers were applied to the upper two inches of soil and allowed to percolate into the soil by several weeks' watering. After this treatment the plants were measured and weighed and set in the jars. Before weighing the roots were cut to 10 inches in length.

In series III blocks of the nursery soil, in which were 1-0 white pine seedlings, were taken from the nursery bed, trimmed down to fit the 1-gallon jars, and the fertilizers applied to the surface. In series IV, in which jack pine seed was used, the fertilizers were worked into the upper two inches. When the jack pine seedlings were up and well established they were thinned to about 25 per jar. Distilled water was applied to the jars every three or four days.

When the final weights and measurements were taken in May, it was found that practically no height growth had taken place in any of the series, although those which responded to the treatment showed distinct

TABLE 3. *Effect of fertilizers on transplants of Norway pine and white spruce* (Series I and II)

Fertilizer	Gms. per jar	Pounds per acre	Norway pine, series I: Total weight seedlings grams 1930	Norway pine, series I: Total weight seedlings grams 1931	Norway pine, series I: Increase pctg.	White spruce, series II: Total weight seedlings grams 1930	White spruce, series II: Total weight seedlings grams 1931	White spruce, series II: Increase pctg.
Concentrated	3.12	500	8.33	10.82	30	3.90	5.60	44
tankage	6.24	1,000	10.33	12.35	20	4.43	6.35	43
Steamed	1.87	300	12.60	15.82	26	3.50	5.27	51
bone meal	3.74	600	8.60	12.05	40	4.77	5.82	22
2-1-1	1.25	200						
superphosphate	.624	100						
plus	.208	33	16.34	22.85	40	3.67	4.60	25
muriate potash	2.50	400						
sodium nitrate	1.25	200						
	.416	66	10.75	17.22	60	3.44	4.45	29
Ammonium	.97	155	12.47	17.23	38	2.78	3.80	37
sulfate	1.94	310	10.32	11.80	14	2.65	3.80	43
Superphosphate	.75	120	20.68	27.10	31	2.92	4.05	39
	1.50	240	16.53	23.67	43	2.10	2.85	36
Muriate potash	.62	100						
plus	.75	120	14.31	21.52	50	2.40	3.70	54
superphosphate	1.25	200						
	1.50	240	16.91	21.25	26	1.91	3.00	57
Ammonium sulfate	.97	155						
plus	.75	120	14.96	23.98	60	3.08	4.32	40
superphosphate	1.94	310						
	1.50	240	13.67	20.19	48	3.07	3.75	22
Check	....	....	24.93	31.60	27	5.67	7.00	24

increase in the fullness of the tops and bushier crowns. However, a very distinct difference in root development between the fertilized and unfertilized stock was revealed, and the results give strong proof that root growth of conifers raised in the greenhouse, or in the nursery where the soil temperatures are suitable, may begin several months earlier than the tops. At the end of the experiment some roots were 24 inches long, although they had been cut to a length of 10 inches when installed in the fall.

In series I and II we find that the 2-1-1 combination of superphosphate, muriate of potash and sodium nitrate gave an increase of 40 per cent over the checks for Norway pine. This combination without the nitrate gave 50 and 26 per cent increase, somewhat better for the spruce than for

TABLE 4. *Effect of fertilizers on 1-0 white pine and seedlings of jack pine* (Series III and IV)

	Series III, 1-0 white pine			Series IV, jack pine seed	
	Pounds per acre	Total weight of seedlings grams	Rating	Total weight of seedlings grams	Rating
Check		2.30	100	4.0	100
Concentrated	1,500	2.40	100	4.33	108
tankage	3,000	2.12	88	3.55	89
Steamed	900	3.20	137	3.75	96
bone meal	1,800	2.80	117	5.66	142
Sodium nitrate	600				
superphosphate	300				
muriate potash	99	1.90	79	3.00	75
2-1-1	1,200				
ratio	600				
	198	2.25	94	4.12	103
Ammonium	485	2.50	104	4.50	113
sulfate	930	2.25	94	3.75	94
Superphosphate	360	4.25	177	4.30	108
	720	4.85	202	7.00	175
Muriate of potash	300				
plus	360	2.45	102	5.70	143
superphosphate	600				
	720	2.80	117	9.00	225
Ammonium sulfate	465				
plus	360	2.15	90	6.90	173
superphosphate	930				
	720	2.35	98	4.00	100

the pine. (Results for the light applications are stated first and the heavy last.) Superphosphate singly gave 31 and 43 per cent increase for the pine; 39 and 36 per cent for the spruce. The checks show 27 per cent increase for pine and 24 for spruce. Aluminum sulfate and superphosphate also gave very good results with 60 and 48 per cent increase in weight for the pine and 40 and 22 for the spruce. Stoeckler did not go into the matter of top measurements in the nitrogenous fertilizers, thinking it not worth while since no top growth was observed, yet the photographs indicate a very decided increase in the fullness of the tops.

For the white pine in series III the applications of superphosphate ranked first with 177 per cent increase for the light and 202 per cent for the heavy treatment. (100 is used for the checks.) Steamed bone meal proved next best with 137 and 117 (light and heavy use), while the muriate of potash and superphosphate came in third with 102 and 117. These figures are based on the total weights of the seedlings after the roots had been trimmed to 10 inches below the soil surface. In the case of jack pine, which had been raised from seed in the greenhouse, the muriate of potash and superphosphate combination came in first with 143 and 225 per cent increase and the superphosphate second with 108 and 175.

There is some objection to tests with fertilizers run in the greenhouse on the ground that the soil in the jars may be less satisfactorily aerated

than is the case in the nursery, that the circulation of the carbon dioxide and nitrogen is somewhat impeded which through the decomposition of the organic fertilizers play an important role in dissolving certain plant foods. For these reasons the fertilizers which require oxygen for decomposition or carbon dioxide for solubility may never be able to bring out their full values. On this account the results obtained in the greenhouses may not apply in the nurseries.

Bateman, Gray and McComb (2) tested the effects of certain commercial fertilizers on coniferous nursery stock in 1932 at the State Avenue nursery. They used northern white pine and red pine and Norway spruce in seed beds, 1-0 and 2-0 transplanted stock. Seven different fertilizers were used, each with light, medium and heavy application; Urea, urea and lime, ammonium sulfate, dried blood and bonemeal, 1:2, Sheepo, 4-12-0, Vigoro and Vigoro plus 4-12-4. (See table 5.) The seedbeds were 3 x 12 feet divided into four 3 x 3 sections, each section separated by a 1″ x 6″ board placed on edge. The seeded plots were previously treated with formaldehyde at the rate of 5 gallons of 1 per cent solution per nine square feet of surface.

In the case of the transplants there were 55 trees of 1-0 and 44 of the

TABLE 5. *Effect of fertilizers on white pine seedlings, 1-0 and 2-0 transplants*

Fertilizer	Amount per plot grams	Seeds planted in fertilized beds: Average length cm. Tops	Roots	1-0 transplants: Average length cm. Tops	Roots	2-0 transplants: Average length cm. Tops	Roots	Stem diam. mm.
Urea	12.502	9.6	17.7	9.4	28.0	18.4	38.1	6.6
	18.753	10.6	20.7	8.6	24.1	21.5	33.9	6.6
	Control	10.5	20.7	8.2	26.3	16.8	33.3	6.6
Ammonium sulfate	28.115	10.9	20.5	9.0	26.2	17.6	35.4	6.3
	42.173	9.8	20.1	9.9	32.5	12.5	34.4	4.9
	Control	9.8	20.2	10.3	33.1	15.3	29.9	5.6
Urea and lime	12.50- 749.75	7.9	19.9	10.7	19.8	22.1	31.4	8.5
	18.75-1124.63	7.9	16.2	10.4	32.0	18.6	28.4	7.4
	Control	8.7	21.8	9.5	28.2	17.4	30.2	6.6
Dried blood and bone meal	52.351-38.781	10.3	22.7	9.5	21.2	18.8	36.2	7.1
	78.527-58.171	9.1	22.3	9.8	15.3	17.9	29.9	6.7
	Control	9.3	15.4	9.1	30.6	17.3	31.6	6.9
Sheepo	374.87	7.7	14.9	9.4	31.1	16.2	32.9	6.5
	562.31	8.8	16.9	10.2	25.7	19.2	38.8	7.5
	Control	11.5	21.0	9.1	26.8	16.6	28.6	6.9
4-12-0	140.58	8.6	23.2	...	...	21.6	34.0	8.1
	210.87	7.2	23.0	10.2	30.5	23.3	32.1	7.9
	Control	8.1	22.9	10.3	23.2	17.4	28.4	6.2
Vigoro 4-12-4	140.58	8.9	19.6	...	...	18.6	35.6	7.1
	210.87	8.8	22.8	9.9	25.4	18.1	32.5	6.7
	Control	9.8	23.2	8.4	20.8	20.8	32.9	7.2

2-0. The fertilizers were weighed out and applied to the upper two inches of soil. The dried blood and bone meal nitrogen was on the basis of 30, 60 and 90 grams and for phosphorus 60, 120 and 180. The dried blood contained 10 per cent nitrogen and bone meal, 1 per cent phosphorus and 29 per cent potassium. The Sheepo was rated at 1.5 per cent nitrogen and 0.7 per cent P_2O_5. The superphosphate had 20 per cent phosphorus.

Analysis of the nursery soil, made in triplicate, showed 50.8 to 52.4 per cent sand; 12.4 to 20.4 per cent silt, and 20.0 to 27.2 per cent clay; an hygroscopic coefficient 4.40 to 5.57; capillary capacity 45.9 to 55.6; carbon 1.288 to 1.636; organic matter 2.220 to 2.826; pH of 5.23 to 5.38; nitrogen 0.131 to 1.154 per cent and P_2O_5 from 0.0535 to 0.0580 per cent.

Measurements of the height of stem, length of roots and diameter of the stem at ground line were recorded at the end of the first and second years. The plants were then cut in two and the tops and roots dried separately in an oven at 105 degrees C. to a constant weight. The dried samples were weighed and the averages computed for each of the fertilizers used. (McComb did this the first year and Bateman the second.)

Somehow this experiment did not give satisfactory results. There were considerable variations in the results and a lack of definite tendencies expressed. In some cases the transplant survival was deemed insufficient for reliable data, especially in case of the spruce. For these reasons most of the original tabular data have been omitted.

Increase in top and root growth gives no consistent relation for the seedlings, 1-0 and 2-0 transplants throughout, nor were the two species affected in the same direction by the same fertilizer.

Considering the white pine in table 5 it is observed that urea stimulated top growth of the 2-0 stock and caused no effect on the rest; ammonium sulfate produced no general effect but decreased the tops and increased the roots of the 2-0 appreciably. The effect of urea and lime was generally to reduce root development with no top stimulation. Dried blood and bone meal tended to increase the top-root ratio. Results with the Sheepo are negligible except in one instance where a remarkable boost showed in root development of the 2-0 stock, especially with the heavy application. The 4-12-4 was ineffective on the seedlings but showed some stimulation in the tops and roots of the 2-0 white pine. Vigoro and 4-12-4 appears to have augmented both tops and roots of the 1-0 during the first and second seasons.

The fertilizers which evidently became almost immediately available for white pine are ammonium sulfate, 4-12-0 and 4-12-4. Sheepo, on the other hand, is of much slower action and would be more suitable for use on stock which remains longer in the nursery. In general it may be said that, since the soil used in these tests is in itself fairly fertile, differences induced by the use of fertilizers would tend to diminish, as they have in these experiments.

Anderson (1) conducted his experiments in 1933 in the State Avenue nursery at Ames on first year white pine seedlings.

The various fertilizers used are given in table 6. Each of the 4 x 12 nursery beds was divided into three sub-sections designed for individual treatment. The soils were of the Carrington and Dickinson loam. These are quite similar in their chemical composition, but the Dickinson contains more of the sand and shows therefore 6 per cent lower field moisture.

During the growing season the soil moisture was maintained at from

TABLE 6. *Effect of fertilizers on 1-0 white pine seedlings*

Fertilizer	Height of stem cm.	Root length cm.	Oven-dry weight 100 plants			Mineral absorption			
			Tops gm.	Roots gm.	Total gm.	N percentage		P percentage	
						Tops	Roots	Tops	Roots
Check	6.18	23.12	9.80	5.35	15.15	1.30	1.16	.650	.641
Sodium nitrate	6.03	19.92	9.65	4.85	14.50	1.35	1.09	.561	.706
Ammonium sulfate	6.16	22.38	10.23	5.53	15.76	1.70	1.22	.574	.607
Dried blood..	6.26	23.14	11.14	5.13	16.27	1.77	1.30	.644	.687
Superphosphate..	6.17	25.82	11.14	5.80	16.94	1.60	1.06	.650	.743
Muriate of potash	6.69	24.47	10.00	5.26	15.26	1.16	.92	.542	.632
Bone meal ..	5.85	24.25	10.33	5.33	15.66	1.47	1.05	.662	.708
Ammonium sulfate + superphosphate..	6.35	25.40	12.00	7.00	19.00	1.89	1.37	.662	.671
Ammonium sulfate + muriate of potash	6.41	21.99	10.35	5.00	15.35	1.86	1.47	.575	.644
Superphosphate + muriate of potash	6.15	24.93	12.13	5.40	17.53	1.47	1.13	.673	.731
Superphosphate + ammonium sulfate	6.85	24.27	10.85	5.50	16.35	1.70	1.35	.598	.706

40 to 60 per cent of the capillary capacity. Soil analysis previous to the installations give: sand 50.8 to 66.8 per cent; silt 12.4 to 20.4 per cent; clay 20.8 to 29.4 per cent; hygroscopic coefficient 4.40 to 5.57 per cent; field moisture 14.85 to 20.57 per cent; capillary capacity 45.9 to 55.6 per cent; carbon 1.28 to 1.63 per cent; organic matter 2.22 to 2.82 per cent; pH 5.23 to 5.38; nitrogen 0.131 to 0.154 per cent; P_2O_5 0.053 to 0.059.

In these experiments 20 per cent of the superphosphate and steamed bone meal is equivalent to 160 pounds of available phosphorus per acre. Both the phosphorus and the potassium when added to the soil may become unavailable due to several possible combinations. Soil tests which were made subsequent to the application of the fertilizers indicate that differences which may have been brought about by the treatment would not be over 160 pounds per acre, an amount or amounts difficult to distinguish by ordinary methods of testing.

Of considerable interest in this connection are tests which Anderson made of the mineral absorption by the tops and roots subsequent to the

measurements and weights. These may help explain the relative values of the results and throw light on their availability.

Anderson found only slight differences in growth between the treated and the untreated stock at the end of the first summer. (Measurements and weight which were obtained at the end of the second season may come to light later.) He believed this due to the relatively high fertility of the soil itself before application of the fertilizers. The seedlings themselves remove only a small portion of the nutrients, especially 1-0 stock; the results which appear insignificant at the end of the first growing season may become important at the end of the second.

In these tests it was observed that sodium nitrate and ammonium sulfate showed very little effect. Perhaps the sodium nitrate should have been applied after, instead of before the germination. The nitrogen seems to have increased top growth in most cases, especially when it was combined with ammonium sulfate, superphosphate or muriate of potash. (Some agency in the soil may have rendered the nitrogen unavailable.) Even the steamed bone meal treatment remained ineffective, but whenever superphosphate was used, either singly or in combination, a distinct increase in weight was observed, and more so in the roots than tops. Ammonium sulfate and superphosphate in combination gave the greatest increase in weight. Anderson stated that this gain is most likely caused by the superphosphate since the ammonium sulfate singly gave only slight benefit. Muriate of potash and superphosphate in combination also produced better and heavier stock.

By scanning the data in table 6 we may observe that the absorption of nitrogen by the tops was increased mostly by the use of nitrogen fertilizers, especially by the ammonia compounds; results with the dried blood and sodium nitrate were intermediate and low for muriate of potash. The absorption of nitrogen by the roots was highest with ammonia compounds, medium with superphosphate and bonemeal and lowest with the muriate. The use of the phosphorus showed but slight increase of this mineral in the roots and no appreciable increase in the tops.

Anderson stated that phosphorus and potassium may form combinations in the soil and thus become unavailable to the plants. In this connection the following from Nemec (7) are of interest: "In some instances favorable results were obtained in height growth of evergreen seedlings in the nursery with 40 per cent potassium salts but only if the soils were neutral or alkaline and contained an insufficient amount of soluble potassium (less than 160 miligrams of K_2O soluble in 1 per cent citric acid). In a soil group with an acid reaction indicative of a higher chlorine content the fertilizing with salts of potassium indicated no increase in growth of the pine, even in the case where the soil lacked potassium to a large extent. The retarding influence of the 40 per cent potassium salts would not result from the K_2O content but from the unfavorable influence of the chlorine in the potassium salts. The fertilization with potassium sulfate was not attended with favorable results. Fertilization with potassium has in most cases shown a decrease in the absorption of this element in the pine needles."

SUMMARY

Tests were made of the effect of organic and inorganic fertilizers on evergreen forest planting stock at Ames, Iowa, by several graduate stu-

dents in the Department of Forestry. Some of the more outstanding results are as follows: On a somewhat alkaline soil the aluminum sulfate, sodium nitrate and 2-12-6 definitely increased top growth of white pine; aluminum sulfate stimulated root development of white pine and Douglas fir; superphosphate and Vigoro benefitted both tops and roots of the Norway spruce. (Marriage.)

In the greenhouse tests the rotted manure, superphosphate and dried blood stimulated top growth of white pine but dried blood, with muriate of potash plus superphosphate and Nitrophoska gave increased growth and good top-root balance. For Norway pine the sodium nitrate and muriate of potash gave marked increase in weight but there was no response with dried blood alone. (Christensen.)

Tests with Norway pine and white spruce in the greenhouse responded well to superphosphate, muriate of potash and sodium nitrate in combination; the nitrate, however, did not seem essential for the spruce. Both the pine and the spruce responded well to treatment with superphosphate and aluminum sulfate. In case of white pine and jack pine a combination of superphosphate, steamed bone meal or superphosphate with muriate of potash gave good results. (Stoeckler.)

Bateman, Gray and McComb obtained increased top and root growth of white pine in the nursery with Vigoro and 4-12-0; increase in tops over the roots with dried blood and bone meal; Sheepo and 4-12-4 appeared rather slow acting for one year white pine stock.

In another set of experiments carried out in the nursery, superphosphate increased the weights of 1-0 white pine very materially, especially the root parts; some response was also noted with sodium nitrate when combined with superphosphate or ammonium sulfate. Steamed bone meal remained ineffective.

The absorption of nitrogen by the leaves was augmented by the ammonia compounds, superphosphate, dried blood and sodium nitrate, and the superphosphate, bone meal and muriate of potash combination tended to increase the absorption of phosphorus in the roots. (Anderson.)

In general the use of nitrogen fertilizers gave increase in top growth of the seedlings and transplants and those containing phophorus definitely increased root development. In the nursery a combination of fertilizers containing nitrogen, phosphorus and potassium was in most instances more effective than a single treatment of only one element, both in the organic and inorganic applications. In the greenhouse tests single elements frequently proved more effective.

LITERATURE CITED

1. Anderson, C. E.
1933. The effect of commercial fertilizers on white pine seedling stock. MSS. Iowa State College of Agriculture and Mechanic Arts. 73 pp. illus.

2. Bateman, B. A., A. L. McComb, and Gordon Gray
1934. MSS. reports on nursery fertilizer experiments, Dept. of Forestry, Iowa State College of Agric. and Mechanic Arts.

3. Christensen, I. L.
1931. Investigations of forest nursery practice in the United States. MSS. Iowa State College of Agric. and Mechanic Arts.

4. Lunt, H. A.
1938. The use of fertilizers in the coniferous nursery. Conn. Agric. Expt. Sta. Bull. 416, 33 pp. illus.

5. Marriage, Lester
1932. The use of chemical fertilizers for coniferous seedlings in the forest nursery. MSS. Dept. of Forestry, Iowa State College of Agric. and Mechanic Arts. 12 pp. illus.

6. Mertz, A. R.
1936. Selecting fertilizers. U. S. Dept. of Agric. Circular 487.

7. Nemec, Antonin
1932. Etud sur la tenur du sol en chlore et sur l'action des engrais potasique sur la croissance du pin dans les pepinieres foretieres. Revue des Eaux et Forets, **70**(5): 375-379.

8. Stoeckler, J. H.
1931. Uses of fertilizers for forest trees with special reference to nursery practice. MSS. Iowa State College of Agric. and Mechanic Arts. 77 pp. illus.

9. Petersen, J. B., F. B. Smith, and P. E. Brown
1937. Effect of phosphate fertilizers on the reaction of Grundy silt loam in greenhouse experiments. Iowa State Jour. Sci. **11**: 3;293-309.

10. Pierre, W. H.
1934. Effect of Mono-, di-, and tricalcium phosphates on the reaction of soils of different degrees of acidity. Jour. Amer. Soc. Agron. **26**: 278-289.

11. Wahlenburg, W. G.
1930. Experiments in the use of fertilizers in growing forest planting material at the Savanac Nursery. U. S. Dept. of Agric. Circular 125; 37 pp. illus.

PLATE I.

FIG. 1. (upper) Two-year-old white pine seedlings, fertilized with aluminum sulfate.
D—Two hundred pounds per acre.
F—Two hundred fifty pounds per acre.
E—Control.

FIG. 2. (center) Two-year-old white pine seedlings.
D—Fertilized with Vigoro at 250 pounds per acre.
F—Fertilized with superphosphate at 200 pounds per acre.
E—Control.

FIG. 3. (lower) Two-year-old white pine seedlings.
D—Fertilized with ammonium sulfate at 150 pounds per acre.
F—Sodium nitrate at 200 pounds per acre.
E—Control.

PLATE I.

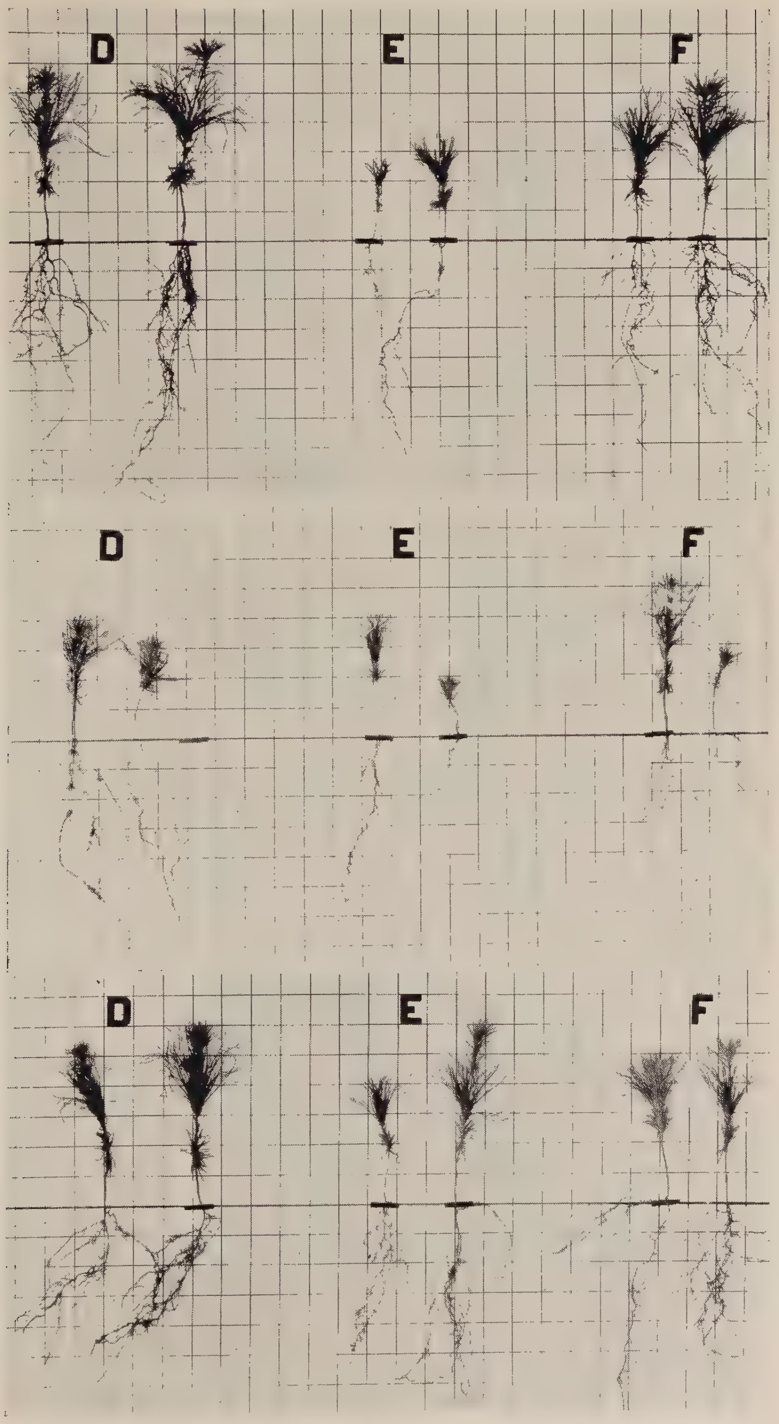


PLATE II.

FIG. 1. (upper) Norway pine in greenhouse fertilizer experiments.
D—Muriate of potash.
F.—Superphosphate.
E—Control.

FIG. 2. (lower) Norway pine in greenhouse fertilizer experiments.
A—Ammonium sulfate.
C—Superphosphate.
B—Control.

PLATE II.

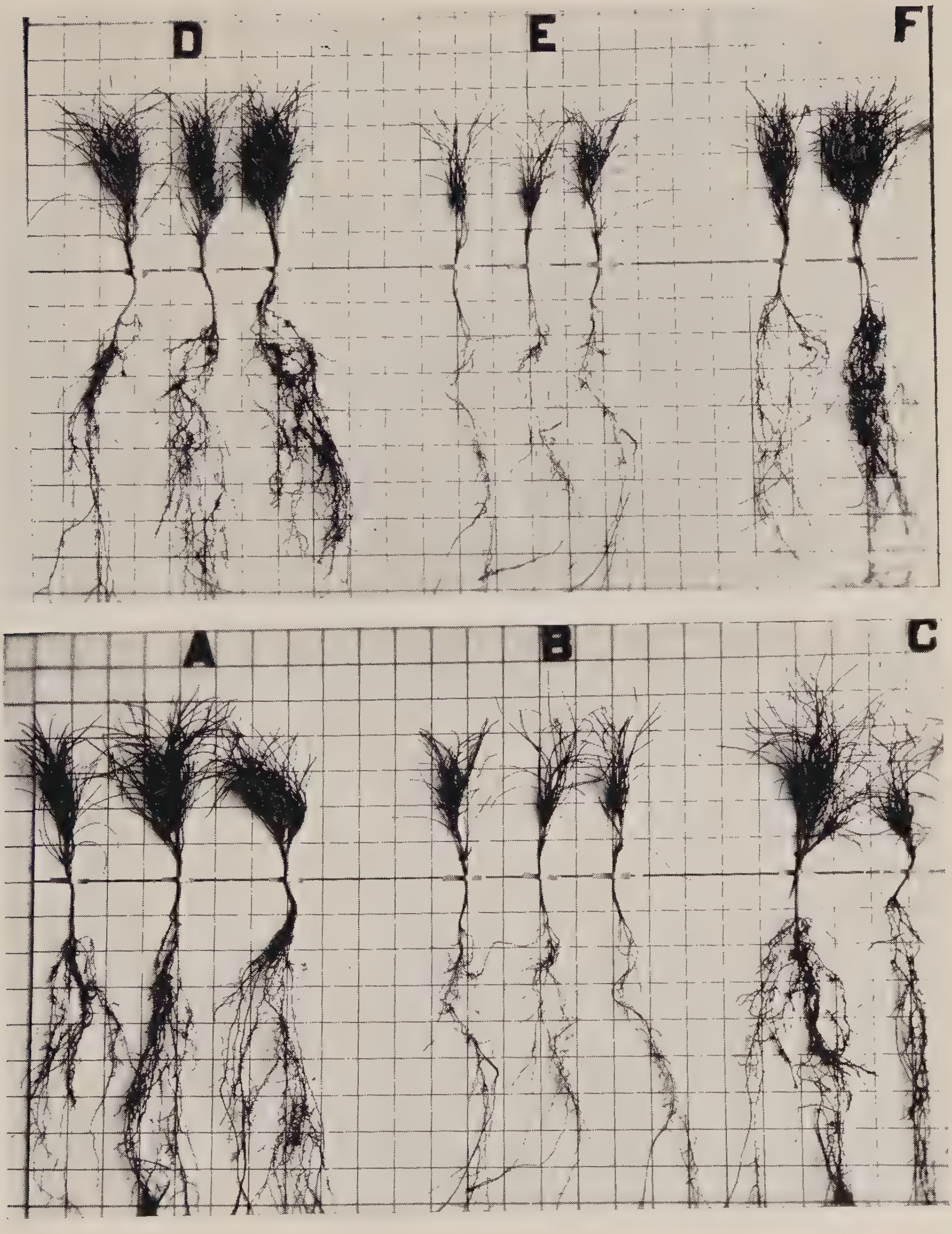
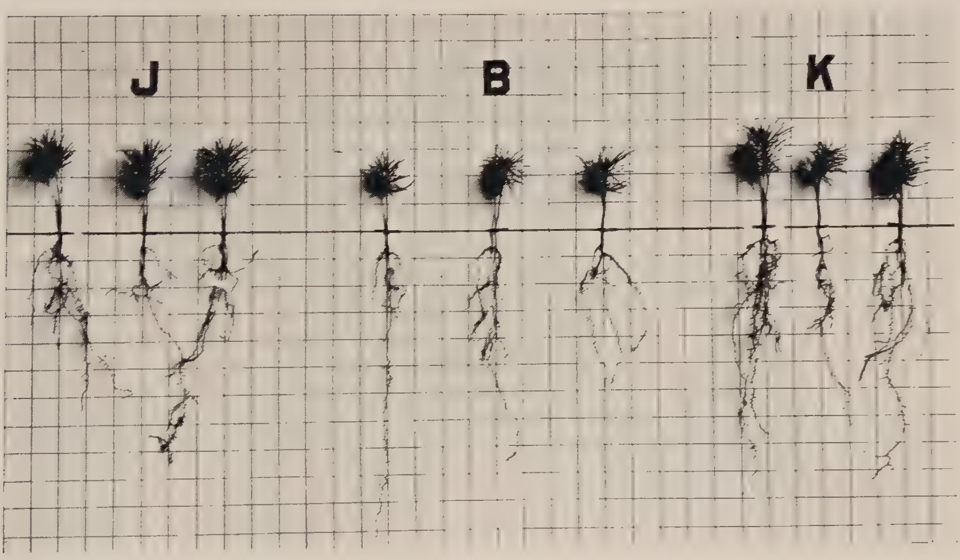


PLATE III.

One-year-old white pine in nursery fertilization experiments.

J—Superphosphate and muriate of potash at 800 and 160 pounds per acre, respectively.
B—Control.
K—Ammonium sulfate and superphosphate at 400 and 800 pounds per acre, respectively.

PLATE III.



ACTION OF MICRO-ORGANISMS ON FATS

I. THE SIGNIFICANCE OF COLOR CHANGES IN DYES USED FOR THE DETECTION OF MICROBIAL ACTION ON FAT

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Relatively little is known concerning the action of micro-organisms on fats. Most of the tests now available for practical analytical work give only a general indication of the nature of the changes involved. This applies particularly to those methods where the action of micro-organisms is determined by the changes they produce in globules of fatty material suspended in an agar medium. The nile blue technique used by Turner (1), the copper sulphate method employed by Carnot and Mauban (2) and Berry (3), and the simple triglyceride and natural fat techniques developed by Hammer and his associates (4), all give evidence of some action of the bacteria on the fat with the probable production of free fatty acids. It is obvious, however, to those familiar with these methods, that the reactions they demonstrate are more complex than simple hydrolysis of the triglycerides to form free fatty acids. The term lipolytic as applied to micro-organisms, and as determined by the results of these and similar tests, is capable of wider interpretation.

As shown by Collins and Hammer (5) various lipolytic bacteria differ in their action on simple triglycerides. The fact that an organism produces free fatty acid from tripropionin or tributyrin is no indication that the same organism will hydrolyze mixed triglycerides of a natural fat, such as butter, lard, or cottonseed oil.

A factor further complicating the situation, and not taken into consideration in these tests, is that many organisms producing enzymes which hydrolyze the fats, also produce powerful oxidases. It is conceivable that acids formed during the later stages of oxidation may give with these tests a result similar to the action of a simple lipase. In most cases, however, hydrolysis and oxidation will be proceeding at the same time, producing the complex and varied reactions which are known to be taking place.

From a practical standpoint, when the "lipolytic" bacteria are further classified by their actions on fats, a step will be made towards better understanding of the relation between bacteria and many of the off flavors they produce in fatty foods.

The object of the work reported in this paper is to throw further light on the significance of color changes in certain dyes which may be used as indicators in fat emulsion agar media. Nile blue sulphate was used because of its apparent suitability as an indicator for this purpose, as well as the fact that it has been recommended by the Committee on Bacteriological Methods of the American Dairy Science Association (6). So far as is known, methylene blue has not been used to show changes in fat. Its widespread use in so many other ways and the general familiarity

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with its mechanism lends added interest to its application as an indicator of bacterial action on fat. p-Aminodimethylaniline monohydrochloride has been recommended by Jensen and Grettie (7) to be used in conjunction with oil emulsion agars for detecting the action of microorganisms on fats. They first grow the organism in an oil-agar medium without the addition of any indicator. Lipolytic bacteria are identified by a transparent or translucent zone around the colonies. This oxidase-detecting dye is then flooded over the surface of the agar and those colonies producing oxidizing enzymes are indicated by the deep rose-red or purple colors they assume. Further observation with the microscope shows that as well as the bacterial colonies the fat globules themselves take on various colors when this dye is added. In the first case, the color results from the direct action of the bacterial enzymes on the dye. In the second case, the dye shows, not the direct action of the enzyme, but the apparent changes the enzymes produce in the fat.

NILE BLUE SULPHATE

The peculiar staining properties of nile blue sulphate and other dyes of the oxazine series were discovered by Smith and White (8) of Manchester University. At their request Thorpe (9) undertook to investigate the chemistry of the phenomenon. The conclusions of this early work are as follows: Applied to material containing neutral fat and fatty acids, these dyes colored the fat red and the fatty acids blue. In reality it is a double dye, the oxazine, and its corresponding oxazone produced from it by boiling in dilute sulphuric acid. Fats and many solvents (xylol, ether, petroleum ether, benzine, chloroform, carbon tetrachloride, and carbon disulphide) take up only the oxazone. Fatty acids take up both dyes and are colored from blue to purple, depending upon the relative amounts of blue and red substances present in the dye. An aqueous solution contains salts of both these bases, and the red oxazone can be increased *ad libitum* by boiling the solution with dilute sulphuric acid. This reaction is exhibited only by those dyes which are derivatives of phenonaphthoxazine. The production of the red compound results from the replacement of the amino- or substituted amino-group by hydroxyl and the consequent formation by intramolecular change of the corresponding phenonaphthoxazine.

In 1928 Turner (1) used nile blue sulphate as an indicator for lipolytic bacteria grown on an oil emulsion agar, and by comparison found it greatly superior to other methods. He also found the dye had properties of a pH indicator, changing from blue to cherry red at about 7.3 to 7.5. Later, Clark (10) gave "blue 10.2-13.0 rose" as the pH range of nile blue sulphate (nile blue A), and "blue 7.2-8.6 rose" as the pH range of a similar dye, nile blue chloride (nile blue 2B). This may explain the discrepancy in Turner's pH range for nile blue sulphate.

Collins and Hammer (5) studied the action of nile blue sulphate on various fats and fatty acids. They found tripropionin, tributyrin, tricaproin, tricaprylin, and triolein were all colored bright red; tricaprin, trilaurin, trimyristin, tripalmitin, and tristearin were all colored red to a degree which decreased rapidly with the increase in melting points until very little red color was present. Butyric, caproic, caprylic, and oleic acids were colored uniformly blue; capric and lauric acids varied in the intensity of blue, while myristic, palmitic, and stearic acids absorbed very little

of the blue color. Beef tallow, butterfat, cocoanut oil, corn oil, cottonseed oil, lard, linseed oil, and olive oil were all colored uniformly bright red.

Jensen and Grettie (7) substituted a mineral oil in place of digestible fats in an agar emulsion, using nile blue sulphate as an indicator, and observed color reactions in colonies similar to those considered lipoclastic on vegetable oil or animal fat. They believe this phenomenon to be due to "the action of bacteria concentrated on the interfacial trap in the oil-water emulsion, acting on the dye in some manner, perhaps changing the oxidation-reduction potential."

In their "studies on Oxidation-Reduction" Cohen and Preisler (11) made an extensive investigation of nile blue sulphate. They found the oxazine to be an Eh indicator, and on the scale of electrode potential, to cover a range between methylene blue and indigo carmine. They made no mention of oxidation-reduction characteristics of the fat soluble oxazone. Apart from this one paper, little has been done to determine the action on nile blue sulphate of changes in the oxidation-reduction potential.

THE PROBLEM

The problem in studying color changes in an oil emulsion agar medium stained with nile blue sulphate is not only the simple phenomenon of red stained fat becoming blue with the formation of free fatty acids. Many substances other than fatty acids are colored blue with the blue base of nile blue sulphate. In the globules the color may be restricted to small portions, or it may diffuse throughout the whole globule. Different organisms vary in the rate they change the red to blue; some become only shades of deeper red or purple; others change completely to blue, and with most of the lipolytic organisms the color entirely disappears from the globules after a few days' incubation. It must also be remembered that from a physical-chemical standpoint the problem is complex. There are two systems, the oil emulsion and the colloidal agar gel, each in intimate contact, each containing a portion and a different portion of the dye. Little, if any, work has been done to determine the action on the two dyes in nile blue sulphate of changes in the oxidation-reduction potential.

To determine some of these facts, after finding the pH range of the aqueous solution, much of this present work will consist of observing the reactions of this dye when separated into fatty and non-fatty solutions, as it is in the actual tests, and not only as an aqueous solution, as most others have considered it.

EXPERIMENTAL

Stock solutions of n.b.s.[2] were prepared by dissolving one gram of "B.D.H. Nile Blue" in one liter of distilled water. To this 4 ml. of concentrated sulphuric acid were added and the solution boiled under a reflux condenser for one hour. Shaken with olive oil or xylol, this gave a deep blue aqueous layer and the xylol or olive oil was deep pink in transmitted light and had a brilliant orange fluorescence in reflected light.

The pH range was determined by adding the dye to a series of buffer solutions and then rechecking the pH of each solution separately on the

[2] In the remainder of the paper nile blue sulphate will be indicated by the letters n.b.s.

potentiometer. The dye concentration in the solutions was 1:20,000. Table 1 shows the results of these readings.

TABLE 1. *pH Range of n.b.s.*

pH	Color
7–10.1	Uniformly blue
10.3	Lighter blue
10.7	Lilac
11.4	Pinkish mauve
11.7	Rose

These results agree with those obtained by Clark (10) who gives the color range of nile blue A. as "blue 10.2–13.0 rose."

Effect of pH on the Color Absorbed from N.B.S. Solution by Olive Oil

Very little is known about the effect of the pH of the aqueous solution on the red color absorbed from it by a fat or oil. In order to determine this a series of pH buffer solutions was made up with a range from about 2 to 12. One ml. of the stock n.b.s. solution was mixed with 10 ml. of the buffer solutions and their pH and Eh readings again checked on the potentiometer. Two ml. of olive oil were added to each tube and after a vigorous shaking these were centrifuged. Table 2 gives the results of these readings.

TABLE 2. *Color changes in olive oil added to a series of n.b.s. solutions of increasing pH values*

Aqueous solution			Color of added oil	Final color of aqueous solution
Color	pH	Eh (volts)		
blue	2.50	.82	very pale pink	very pale blue
blue	2.83	.50	very pale pink	very pale blue
blue	5.17	.45	pale pink	blue
blue	5.63	.41	pale pink	blue
blue	6.00	.39	pink	blue
blue	6.68	.36	red	blue
blue	6.90	.38	red	blue
blue	7.38	.35	purplish red	pale blue
blue	8.60	.32	purplish red	very pale blue
purple	10.90	.23	colorless*	pale blue
mauve	11.65	.22	colorless*	very pale blue

* Oil saponified and all traces of color gone

It would at first appear from this, that up to the point where saponification occurs, an increase in pH of an aqueous n.b.s. solution is accompanied by an increase in the oxazone extractable by oils or fats. However, this is not necessarily so. The change in color from pale pink to deep purplish red may have nothing at all to do with the amount of oxazone

present. As shown in later experiments the pink oxazone in fats acts as an oxidation-reduction indicator, and the change here from pale pink to purplish red is more closely related to the decrease in the oxidation-reduction potential than it is to the increase in pH.

Oxidation of Aqueous N.B.S. Solution

The blue color in fat globules stained with n.b.s. and acted upon by lipolytic bacteria very often disappears after one or two days of incubation. Circumstances indicate that this is probably due to oxidation; consequently, experiments were carried out by oxidizing the dye in various ways.

Continued bubbling of air for five hours through several samples of n.b.s solution at room temperature brought about no color change. Small amounts of ozone, hydrogen peroxide, or exposure to bright sunshine caused decolorization. The addition of olive oil or xylol to samples before and after oxidation showed no increase in the oxazone. However, if oxidation was accompanied by some hydrolytic agent (increased acidity or temperature) there was an apparent increase in the oxazone as shown by the increase in the intensity of the pink color in the added oil or xylol.

Close examination of the solutions as they are being oxidized shows that decolorization is brought about by the precipitation of the dye in the form of very fine, dark suspended particles which settle out on standing. Continued oxidation after their first appearance increases the size of the

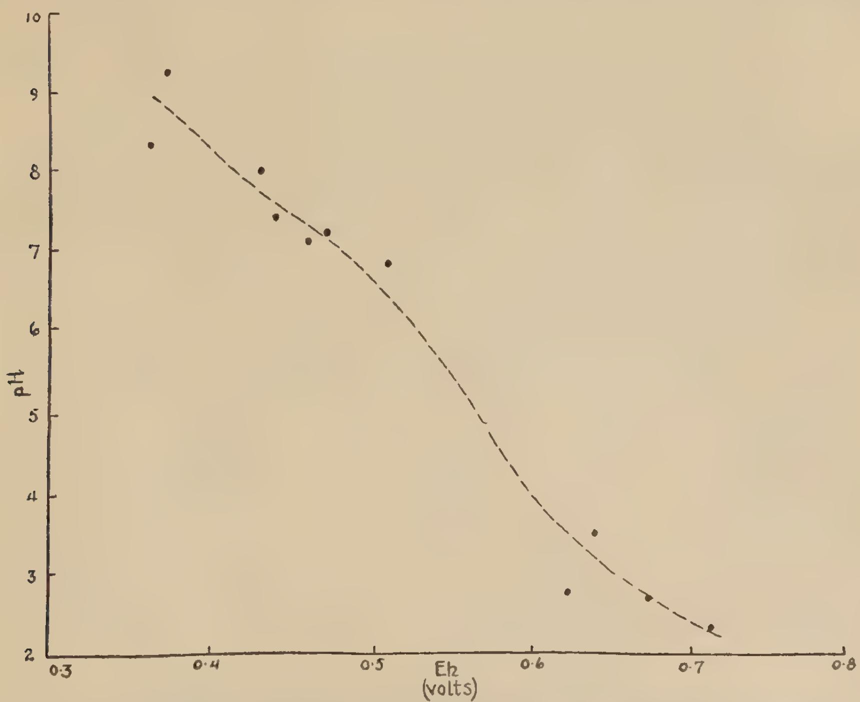

Fig. 1. Eh and pH readings at points where blue begins to fade from buffered solutions of n.b.s. being oxidized with ozone.

particles, and their color changes from blue to purple and then to red. Still further oxidation and the particles disappear entirely.

An attempt was made to find the approximate oxidation-reduction potentials, at various pH values, at which this decolorization or precipitate-formation occurs. A number of buffer solutions having a pH range from 2 to 9 were prepared. To these n.b.s. was added giving a final dye concentration of 1:20,000. A small amount of ozone was slowly bubbled through each solution until the first sign of the fine, dark precipitate appeared. A sample was removed at this point and its pH and Eh measured on the potentiometer. The data obtained are presented in figure 1. It is realized that these figures are only approximations and are not given as the exact Eh measurements at which the color change in n.b.s. occurs. The general trend, however, agrees with what is known to be true about other oxidation-reduction indicators. As the pH is increased, the Eh at which the color change takes place is decreased.

In oxidizing an aqueous solution of n.b.s. it must be remembered that there is also present some of the fat soluble oxazone. A fat extract of this pink dye is also decolorized by exposure to oxidizing agents such as ozone or hydrogen peroxide or the catalytic effect of bright sunlight. If the aqueous solution is oxidized to the point where it becomes colorless and a xylol extract is also colorless, the color change appears to be non-reversible. As an example of this, an aqueous solution was oxidized by exposure to sunlight until the color had completely disappeared. A small portion shaken with xylol left it colorless. This was then divided into three portions and treated as follows:

1. Hydrogen bubbled through it for five hours.
2. Excess sodium bisulphite added.
3. Excess sodium hydrosulphite added.

There was not the slightest color change in any of the samples. Another solution of n.b.s. was buffered at a pH value of 7 with a potassium dihydrogen phosphate-sodium hydroxide buffer mixture. This was then decolorized by slowly bubbling ozone through it. After decolorization hydrogen gas was bubbled through for three hours. As shown in table 3, in spite of the fact that Eh was reduced beyond the point where the solution had been previously colored, it remained colorless.

TABLE 3. *Potentiometer readings of an aqueous n.b.s. solution reduced by hydrogen following oxidation by ozone*

N.b.s. solution	Color	Eh (volts)	pH
Before oxidation	deep blue	.38	7.0
Decolorized by O_3	colorless	.54	7.1
Reduced by H_2	colorless	.11	7.1

This again suggests that oxidation of the oxazine is a non-reversible reaction.

Oxidation of the Red, Fat Soluble Oxazone

Oxidation of the oxazone with ozone, hydrogen peroxide and other oxidizing agents also brings about decolorization. There are some appar-

ent exceptions to this. When the oxazone is extracted in triolein or olive oil and then placed in strong sunlight, it turns to shades of yellow, green, or blue instead of becoming colorless. If it is added to xylol, or some other more stable fat solvent, and then exposed to sunshine it slowly becomes colorless. If the olive oil or triolein extract is treated with ozone or some other oxidizing agent it becomes colorless. The apparent explanation for this is that light is a catalyst and is not in itself an oxidizing agent. As well as the dye, the unsaturated fats present are easily oxidized. Where ozone is added both the oil and the dye are oxidized. Where a catalyst (sunlight) is added without the addition of an oxidixing agent, the dye becomes the donor and is reduced as the fat becomes oxidized. This is further confirmed by the fact that the addition of reducing agents to the oxazone changes it from pink or red to shades of purple, blue, or greenish blue. This is of particular interest from the standpoint of the color changes in fat globules stained with n.b.s. Under certain circumstances the catalytic action of bacterial oxidases on the unsaturated fats in the globule may bring about the reduction of the pink oxazone to shades of blue or purple. The same enzyme action on the same fat in the presence of a more available supply of oxazone may decolorize the dye instead of reducing it.

Addition of Reducing Agents to the Oxazone

From the previous experiments there was an indication that the oxazone of n.b.s. may be reduced from a fat soluble pink or red to a fat soluble blue or purple form. This becomes significant when it is realized that many of the products of fat decomposition (glycerol, certain aldehydes, alcohols, and acids) are relatively strong reducing agents. In order to confirm this, many substances of various reducing capacities were added to the pink oxazone extract and their color reactions recorded.

First, a number of salts and alkalies of various reducing powers were added directly to the stained olive oil. Approximately 0.5 gram of salt was added to 5 ml. of oil. After being shaken, the tubes were left to settle and any color changes noted at the end of five minutes. At the same time about 0.5 gram of the same materials was added to approximately 5 ml. of distilled water and the pH and Eh of these aqueous solutions deter-

TABLE 4. *Color changes in the oxazone of n.b.s. in olive oil produced by the addition of various salts and alkalies, together with the pH and the approximate Eh of these same materials in a corresponding aqueous solution*

Substance added	Color changes in stained oil	Aqueous solution pH	Eh
1. Sodium hydrosulphite	deep blue	`6.5	—.360
2. Sodium bisulphite	blue	6.6	+.030
3. Sodium hydroxide	blue	13.0	+.043
4. Potassium hydroxide	blue	12.6	+.050
5. Potassium nitrite	blue	10.2	+.140
6. Sodium nitrite	?	9.0	+.250
7. Sodium potassium tartarate	no change	8.2	+.290
8. Ferrous ammonium sulphate	no change	3.7	+.310
9. Magnesium sulphate	no change	6.0	+.340
10. Sodium chloride	no change	6.6	+.416

mined. In most cases the Eh was poorly poised and continued to drift slightly. The results, therefore, shown in table 4 must be regarded as simply indicating the general trend. In some of the tubes, after standing 24 hours or more the blue color disappeared.

A second series was prepared including more organic compounds. Instead of measuring electrometrically the potentials set up by aqueous solutions of these substances, their comparative reducing action was observed in an aqueous methylene blue solution. Another difference was that xylol was used in place of olive oil for the oxazone solvent. The results of these observations are seen in table 5.

TABLE 5. *Color changes in xylol solution of nile blue oxazone and in aqueous methylene blue on adding various reducing substances*

Substance added	Color of xylol-oxazone solution	Change in methylene blue
Sodium hydrosulphite	bluish purple	completely reduced
Thymol	bluish purple	no change*
Phenol	purple	partially reduced
Betanaphthol	purple	partially reduced
Naphthol	purple	partially reduced
Indol	purple	partially reduced
Diphenylamine	purple	no change*
Amyl alcohol	mauve	reduced and extracted
Glacial acetic acid	mauve	slightly reduced
Absolute ethyl alcohol	mauve	
Pyrogallol	rose	slightly reduced
Acetone	rose	slightly reduced
Benzoic acid	rose	no change*
Chloroform	rose	no change
Dulcitol	pink	no change
Sorbitol	pink	no change

* Insoluble or very slightly soluble in water.

Aqueous solutions of hydroquinone, glycerol, elon, and ascorbic, citric, malic, acetic, lactic, and tartaric acids all became blue when shaken with solutions of the oxazone in xylol. Concentrated glycerol, lactic, acetic, and citric acids shaken with the xylol extract also turned to various shades of blue. When olive oil was substituted for xylol as the solvent of the oxazone, the results were similar, with slight variations.

Fatty Acids

Added to aqueous n.b.s. the liquid fatty acids readily absorb the dye, which remains unchanged. But added to an olive oil or xylol solution of the oxazone, the pink color is changed to shades varying from rosy pink to mauve. None of the fatty acids was able to change the oxazone from pink to blue as has so often been suggested in literature on this subject. Each fatty acid appears to produce its own peculiar color, and the addition of an excess simply increases the depth of color without changing its shade. The crystals of the higher fatty acids remain colorless in the

presence of oxazone until they are melted, when they too take on various shades of mauve.

Absorption of Aqueous Nile Blue into Fat or Oil from a Surrounding Agar Medium

From the preceding experiments it seems obvious that if the pink oxazone in the globules themselves is the source of the color when bacterial enzymes turn the globules blue, the mechanism must be one of reduction. There is, however, a second method by which globules may turn blue. If, in the decomposition of the fat, a substance is formed in which blue dye is soluble, and which is also fat soluble, this substance may extract the blue from the aqueous to the fatty phase. In order to test this action the following experiment was run: Five ml. of beef extract agar were stained with n.b.s. and put into each of fifteen test tubes and allowed to harden. On top of this was placed either 1 ml. of pure fatty acid or 1 ml. of olive oil containing two drops of fatty acid or some other substance. These were corked and left to stand at room temperature for 24 hours. As shown in table 6, the lower fatty acids, either concentrated or diluted in oil, are able to extract the blue color from the adjacent stained agar. This is very marked in the case of butyric acid, which is completely soluble in water. As the acids become less soluble in water, naturally this power of absorbing the blue dye decreases. It is interesting to note that substances other than fatty acids are able to extract the color in exactly the same manner. Thymol, and amyl and isobutyl alcohols, which are soluble in olive oil, absorbed the blue dye in this way. Lactic acid, which is not fat soluble, absorbed the blue dye, but did not color the oil.

TABLE 6. *Color extracted from beef extract agar stained with n.b.s. by various substances*

Substance added	Color extracted
Olive oil	none
Concentrated lactic acid	deep blue*
Concentrated butyric acid	deep blue
Concentrated caproic acid	pale blue
Concentrated oleic acid	pale blue
10 per cent lactic acid in olive oil	acid blue; oil unchanged
10 per cent butyric acid in olive oil	pale blue
10 per cent caproic acid in olive oil	pale blue?
10 per cent oleic acid in olive oil	very pale blue
10 per cent lauric acid in olive oil	very pale blue
10 per cent myristic acid in olive oil	unchanged
10 per cent palmitic acid in olive oil	unchanged
10 per cent thymol in olive oil	very pale blue
10 per cent isobutyl alcohol in olive oil	very pale blue
10 per cent amyl alcohol in olive oil	very pale blue

* In each case where the added substance became blue there was a noticeable decolorization of the adjacent agar.

DISCUSSION

In most of the previous work with nile blue sulphate this dye has been considered either as a pH indicator (1), a specific test for fatty acids (8), or for the unsaturated fatty acids (13). The observations reported in this paper suggest that there is some need for a revision of our explanation of its mechanism.

It must be realized that n.b.s., as used for detecting lipolytic organisms, is a double dye. The fat in the medium contains only the pink oxazone and the agar may contain both the oxazine and the oxazone. When the fat globules turn blue under the influence of microbial enzymes, the blue color may come from either one of two sources. By reduction the pink oxazone within the globule may be changed to a fat soluble blue; or the blue may be extracted from the adjacent agar medium by the formation within the globule of some substance sufficiently soluble to absorb the dye.

Eisenberg (12), in a recent article on this subject, states, "When a solution of free oxazone in oil is mixed with a fatty acid, such as oleic acid, the red color is changed to blue. This reaction is specific and forms the basis of the following medium." In the experiments reported in this paper none of the fatty acids changed the pink oxazone to blue. At best, they produced shades of mauve; oleic acid produced a distinct rose color. But many substances other than fatty acids did change the oxazone from red to purple or blue. If these substances were soluble in the solvent containing the dye the whole solution changed color. If they were not soluble in the solvent containing the oxazone, they extracted the color and at the same time reduced it to purple or blue. These substances are all reducing agents, and the amount they change the color from red through rose, mauve, purple to blue depends upon their strength as reducing agents. This probably explains the facts observed by Jensen and Grettie (7) that under some circumstances globules turn blue under the influence of bacterial enzymes, even when petroleum oil is substituted for a digestible fat.

The other source of blue in the fat globules is by the formation of some substance which is fat soluble and at the same time will readily absorb the blue oxazine. The lower fatty acids and more especially those which are water-soluble have these characteristics. Experiments demonstrating this have been described. However, this is not specific for fatty acids. Other substances such as isobutyl and amyl alcohols, which are soluble in fat, will absorb the blue from adjacent agar stained with n.b.s. Lactic acid is not soluble in fat, but when mixed with fat and brought in contact with the stained agar the acid alone extracts the blue dye. This probably explains the presence of minute, isolated, blue droplets in globules of fat which can occasionally be observed with certain non-lipolytic bacteria in fat emulsion plates stained with n.b.s.

It seems apparent then that oxidation and reduction may be more important than has heretofore been considered in using and interpreting the nile blue sulphate technique for studying changes brought about by micro-organisms in fats. The possibilities are that most organisms producing blue in fat stained with n.b.s. do so by hydrolyzing the fat, freeing fatty acids which absorb the blue aqueous dye. It is also possible that organisms unable to hydrolyze the fat may produce a blue color in the stained fat globules by means of oxidation-reduction reactions. Considering the number of complex fats and oils used with this dye, and considering the paucity of our knowledge concerning bacterial oxidases, their action on fat and the by-products produced, it seems evident that further investigation is needed before any definite conclusions can be drawn as to the exact significance of color changes in fat stained with this dye.

From a practical standpoint, however, this dye should continue to serve a useful purpse. A positive reaction means that the micro-organisms

are having some destructive action on the fat. Whether or not the finer mechanisms of the reactions can be explained will not alter the offensive flavors such organisms may produce in dairy products or other fatty foods.

METHYLENE BLUE

When an aqueous solution of methylene blue is incorporated into an agar medium containing butter fat, it is reduced to its colorless form during autoclaving. Microscopic examination shows that the fat globules are also free from color. When these plates are inoculated with certain types of bacteria, microscopic examination shows that the fat globules adjacent to these colonies turn blue. This involves the double problem of discovering the method by which the dye is apparently transferred to the fat phase, as well as the mechanism that brings about the reversion to the colored form.

Many of the organisms bringing about this change are among those generally considered to be lipolytic. For that reason experiments were conducted to find the effect of fatty acids, and various other substances on methylene blue. One ml. of 1.5 per cent methylene blue was added to one liter of water. Sufficient sodium acid sulphate was added to decolorize the solution after five minutes boiling. This was then adjusted with sodium hydroxide to a pH of 6.75. Two ml. portions of this solution were transferred to small serological test tubes. Approximately 0.25 to 0.5 ml. of each of the substances enumerated in table 7 was added to each tube and their color changes noted over a period of 24 hours at room temperature. A similar series using the oxidized form of the dye was also prepared.

These results, shown in tables 7 and 8, indicate that olive oil, and at least those triglycerides used, do not absorb the dye from the aqueous solution. Also, that under the conditions in which they were incubated, they produced no color change on either the reduced or oxidized forms of methylene blue when the dye is associated with them in an aqueous solution. That this is not the case with unsaturated fats under conditions which favor oxidation is shown by the fact that, in several tests, change in the color of methylene blue is used as a measure of keeping quality. However, triolein, under conditions more nearly comparable to those in the agar plates incubated in the dark at room temperatures, will not reduce the oxidized form of methylene blue.

The saturated fatty acids, especially those of the lower molecular weights, readily extract methylene blue from the aqueous solution, and if it has been previously reduced, change it back to its oxidized form. Oleic acid, in common with the others, absorbs the dye; it differs from the saturated acids in slowly reducing the methylene blue to its colorless form. This is probably due to the withdrawal of oxygen from the solution as the acid itself is oxidized.

Amyl and isobutyl alcohols act towards methylene blue in a manner similar to the unsaturated fatty acids. They extract the dye from an aqueous solution and, if it is reduced, oxidize it.

Acetic, lactic, citric, and amino succinic acids, which are not fat soluble, oxidize the reduced form of the dye, but do so less completely than the lower fatty acids. Acetone (a ketone) and glycerol act as reducing agents towards methylene blue. Formaldehyde very slowly permits a pale blue color to return to the reduced form of the dye.

TABLE 7. *The oxidizing action of various substances as shown by the formation of a blue color when added to reduced methylene blue*

Substance added	Color of aqueous phase	Color of non-aqueous phase
1. Control	colorless	....
2. Olive oil	colorless	colorless
3. Tricaproin	colorless	colorless
4. Tricaprylin	colorless	colorless
5. Trimyristin	colorless	colorless
6. Tripalmitin	colorless	colorless
7. Glycerol	colorless	....
8. Oleic acid	colorless	colorless
9. Butyric acid	blue	....
10. Caproic acid	almost colorless	blue
11. Caprylic acid	almost colorless	blue
12. Lauric acid	colorless	pale blue
13. Myristic acid	colorless	very pale blue at surface
14. 3 percent hydrogen peroxide	blue	....
15. Sodium chloride	top half blue	....
16. Lactose	colorless?	....
17. Acetone	colorless	....
18. Formaldehyde	pale blue	....
19. Russian oil	colorless	colorless
20. Hydroquinone	colorless	....
21. Trimethylamine	colorless	....
22. 95 per cent alcohol	pale blue at surface	....
23. Amyl alcohol	colorless	blue
24. Sorbitol	colorless	....
25. Dulcitol	colorless	....
26. Isobutyl alcohol	colorless	blue
27. Lactic acid	top half blue	....
28. Acetic acid	top half blue	....
29. Citric acid	top half blue	....
30. Uric acid	colorless	....
31. Amino succinic acid	blue	....

The controls of both the oxidized and reduced dye solutions remained unchanged throughout the duration of these experiments.

DISCUSSION

From the standpoint of explaining the color changes in the fat globules these observations suggest several different things. The fats themselves do not absorb the dye. The color therefore must be due to some by-product of fat decomposition which will both extract the dye from its aqueous solution and at the same time oxidize it. The lower saturated fatty acids have these characteristics. It is probable that in the majority of cases where fat globules turn blue in the presence of reduced methylene blue, the production of free fatty acids is the cause. It is also possible that small amounts of other substances capable of extracting and oxidizing reduced methylene blue might be formed during decomposition of fats by bacteria. Peroxides, and some of the carboxy acids, might be the cause of

TABLE 8. *Color changes following the addition of various substances to solutions of methylene blue in its oxidized forms*

Substance added	Aqueous phase	Non-aqueous phase
Control	blue	....
Olive oil	blue	colorless*
Triolein	blue	colorless
Tricaproin	blue	colorless
Trilaurin	blue	colorless
Glycerol	pale blue	....
Butyric acid	blue	....
Caproic acid	blue	blue
Caprylic acid	blue	blue
Lauric acid	blue	pale blue
Myristic acid	blue	colorless
Palmitic acid	blue	colorless
Oleic acid	blue	blue
Lactic acid	blue	....

* Natural color of oil etc., not considered in these descriptions.

isolated blue droplets in the globules. As they are not fat-soluble the blue they abstract will not diffuse throughout the whole globule but remain in isolated areas.

p-AMINODIMETHYLANILINE MONOHYDROCHLORIDE

Like methylene blue, p-aminodimethylaniline monohydrochloride is an oxidation-reduction indicator. It was originally used by Dietrich and Liebermeister (14) for demonstrating the existence of oxidizing granules in the anthrax bacillus. By incorporating this dye, together with an alkaline solution of a-naphthol, into agar, Schultze (15) produced a pale blue medium which would turn to bluish black when streaked with a heavy suspension of certain oxidizing bacteria. Gordon and McLeod (16) repeated and extended the observations of Schultze, advocating this technique as an aid in differentiating closely related organisms. They attempted to incorporate this reagent in a culture medium but found the amount of dye needed to produce the reaction would inhibit growth. Ellingworth, McLeod, and Gordon (17) investigated the similar use of other diamines and found the tetramethyl compound to be the most sensitive and satisfactory for bacteriological technique. They recommended that colonies be flooded with a 0.5 per cent solution of this dye, which gives the oxidizing colonies a deep bluish-violet color.

Jensen and Grettie (7) combined the use of this dye with oil emulsion agar media for diagnosing the action of bacteria on fats. Physical changes in the globules producing a translucent zone around the colonies, they considered to indicate hydrolysis. The reaction of one of these dyes flooded over the surface of the agar indicated whether or not the colony also produced oxidizing enzymes. In this way they were able to distinguish between those organisms which hydrolyze the fat, those which oxidize it, and those which produce both reactions.

The observations of the investigators mentioned above concerned

color changes in the aqueous solution of the dye coming in contact with the bacterial colony. Microscopic observations on the fat globules of oil-emulsion agar plates flooded with the dimethyl compound show characteristic color changes in the fat globules themselves. They may be red, yellow, brown, black, or colorless. Inasmuch as these changes are the result of the action of the bacterial enzymes on the fat itself, their interpretation may be important in the study of lipolytic organisms.

EXPERIMENTAL

A 0.5 per cent aqueous solution of p-aminodimethylaniline monohydrochloride was prepared and one ml. portions were placed in small serological test tubes. Approximately 0.25 ml. of the various substances enumerated in table 9 were then added to the tubes. These were shaken and allowed to settle. In the case of the solid fats and fatty acids they were observed first in the crystal form and later slowly raised to their melting points. Table 9 shows the results of these observations.

TABLE 9. *Color changes after the addition of various substances to a 0.5 per cent aqueous solution of p-aminodimethylaniline monohydrochloride*

Substance added	Color changes	
	Aqueous solution	Non-aqueous phase
Olive oil	none	none
Triolein	much lighter	light amber
Tricaproin	lighter	very light brown
Tricaprylin	lighter	little change
Trilaurin	none	little change
Trimyristin	none	very light brown
Tripalmitin	none	little change
Tristearin	none	little change
Butyric acid	paler?	none formed
Caproic acid	much paler	almost black
Caprylic acid	much paler	dark brown
Lauric acid	paler	almost black*
Myristic acid	not much change	grayish brown*
Palmitic acid	not much change	grayish brown*
Stearic acid	not much change	grayish brown*
Oleic acid	much lighter	light brown
Glycerol	paler (slowly)	none formed
Xylol	none	colorless
Lactic acid	not much change	none formed
Amyl alcohol	not much change	very dark purple
Absolute methyl alcohol	not much change	none formed
30 per cent hydrogen peroxide	deeper color	none formed
3 per cent hydrogen peroxide	slightly deeper	none formed

* Only after melting.

These observations show that olive oil and at least those triglycerides used either do not absorb this dye from an aqueous solution or do so very slowly. The fatty acids, on the other hand, all absorb the dye from the

aqueous sloution. Butyric acid, which is completely soluble in water, naturally differs from the others in this point.

When this dye is absorbed from an aqueous solution by fatty acids it changes to colors varying from brown to black. The depth of the color varies roughly with the decrease in molecular weight of the acids. It is significant that where butyric acid is mixed with the dye this color change is not noted. As shown by the action of amyl alcohol, some substances other than fatty acids, which are also not water soluble, are able to extract the dye and deepen its color.

Relatively powerful oxidizing agents, such as 30 per cent hydrogen peroxide, when added to the dye solution, increase the depth of the color, and reducing agents such as glycerol reduce the color. But even strong hydrogen peroxide does not increase the depth of the color as much as when it comes in contact with the lower fatty acids that are not completely water soluble.

Similar experiments with the corresponding tetra-methyl compound did not give results similar to those described above.

GENERAL CONCLUSIONS

1. The mechanisms underlying color changes in fat globules in a medium containing any one of the three dyes studied in this paper are not always simple reactions of the same type. Therefore such color changes cannot be used as specific indications of any one type of reaction (i. e. hydrolysis of the fat).

2. The lower fatty acids, either alone or mixed with fat, have the power of extracting these three dyes from an aqueous solution. If the dyes are in a colorless form in the aqueous solution, they are changed to their colored forms after being abstracted by the acids. Some substances other than fatty acids are able to extract the dye from the aqueous to fatty material. It is probable that most of the color changes observed in fat emulsion are due to reactions of this type.

3. In the case of nile blue sulphate, besides extraction of the blue from the aqueous solution by fatty acids or other substances within the globules, similar color changes may be brought about by the reduction of the oxazone from a pink to a blue color. Fatty acids will not reduce the oxazone from pink to blue.

REFERENCES

1. Turner, R. H.
1929. The action of bacteria on fat. Jour. Infect. Dis., 44:126.

2. Carnot, P., and H. Mauban
1918. Réaction colorée de la stéapsin sur la plaques de gelose-graisse emulsionée par production de savon de cuivre. Compt. rend. Soc. de Biol., 81:98.

3. Berry, J. A.
1933. Detection of microbial lipase by copper soap formation. Jour. Bacteriol., 25:433.

4. Long, H. F., and B. W. Hammer
1937. Methods for the detection of lipolysis by microorganisms. Iowa State College Jour. of Science., 11:343-351.

5. Collins, M. A., and B. W. Hammer
1934. The action of certain bacteria on some simple triglycerides and neutral fats, as shown by Nile Blue Sulphate. Jour. Bacteriol., **27**: 473.

6. Committee Reports
1933. Jour. Dairy Sci., **16**: 294.

7. Jensen, L. B., and D. P. Grettie
1937. Action of micro-organisms on fats. Food Research, **2**: 97.

8. Smith, L., and P. White
1907. On the simultaneous staining of neutral fats and fatty acids by oxazine dyes. Jour. Path. and Bacteriol., **12**: 1.

9. Thorpe, J. F.
1907. A reaction of certain coloring matters of the oxazine series. Transactions in the Jour. of Chemical Soc., Lond., **91**: 324.

10. Clark, W. M.
1922. The determination of hydrogen ions. Williams & Wilkins Co., Baltimore.

11. Cohen, B., and P. W. Preisler
1931. Studies on oxidation-reduction. XVI. The oxazines: nile blue, brilliant cresyl blue, methyl capri blue, and ethyl capri blue. Suppl. No. 92 to the U. S. Public Health Reports.

12. Eisenberg, G. M.
1939. A nile blue culture medium for lipolytic micro-organisms. Stain Technology, **14**: (2) 63.

13. Kaufmann, C., and E. Lehmann
1926. Virchows Arch. Path. Anat., 261, p. 623 (quoted by Turner, see reference No. 1).

14. Dietrich, A., and G. Liebermeister
1902. Centr. f. Bakt. Orig., **32**: 858.

15. Schultze, H.
1910. Über eine neue methode zum nachweis von reduktions-und oxydationswirkungen. Centr. f. Bakt. Abt. 1, **56**: 544.

16. Gordon, J., and J. W. McLeod
1928. The practical application of direct oxidase reaction in bacteriology. Jour. Path. and Bacteriol., **31**: 185.

17. Ellingworth, S., J. W. McLeod, and J. Gordon
1929. Further observations on the oxidation by bacteria of compounds of the para-phenylene diamine series. Jour. Path. and Bacteriol., **32**: 173.

A STUDY OF CHOLESTERILENE[1]

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In our investigation of the dehydration of cholesterol (1, 2) several points relevant to a better understanding of the problem were studied. If the specific rotation of cholesterilene (3,5-cholestadiene (2)) is to be considered the highest negative rotation observed (—123.23 (2)), then the samples of cholesteriline of lower negative specific rotation prepared by various methods (1) must be impure. A study of the preparation of cholesterilene by the copper sulfate method demonstrated the point that cholesterilene possessing the highest negative rotation attainable on purification was obtained from the initial crude product having the highest negative rotation. Thus the method of preparation was found to be of more importance than the subsequent purification of the product obtained in order to produce the compound of highest purity. It was also indicated that cholesterilene possessing the more negative specific rotation was obtained at the lower reaction temperatures used although the time necessary for the dehydration was increased. A decrease in the conditions favorable for pyrolytic side reactions was found to be of importance since the impurities in crude cholesterilene are difficult to remove.

Cholesterilene obtained by the zinc dust distillation of cholesterol has been reported (3) to have the following constants during purification: m.p. 68°, $(\alpha)_D + 1.45°$; m.p. 73°, $(\alpha)_D - 53.37°$; and m.p. 75°, $(\alpha)_D - 4.49°$. On the basis of this initial positive specific rotation, it was suggested (5) that the product obtained by the zinc dust distillation of cholesterol was a mixture of cholesterilene of high negative specific rotation and (dextrorotatory) 2,4 cholestadiene. Cholesterilene was prepared according to the method of Fantl (3) but a specific rotation more negative than — 60° was not observed for the samples obtained which had been purified by repeated recrystallizations.

A mixture of cholesterol and zinc dust was heated under reduced pressure and the reaction product was separated into a dextrorotatory alcohol insoluble fraction which was unaffected by treatment with alcoholic hydrochloric acid and weakly dextrorotatory alcohol soluble needles which yielded strongly laevorotatory needles when treated with alcoholic hydrochloric acid. The conversion of the weakly dextrorotatory alcohol soluble product into a strongly laevorotatory compound (cholesterilene) indicated that this product was a mixture of cholesterilene and 2,4-cholestadiene since 2,4-cholestadiene (dextrorotatory) is converted in cholesterilene (laevorotatory) by treatment with alcoholic hydrochloric acid (5) and since it could not have been just 2,4 cholestadiene ($(\alpha)_D + 168.5°$ (4)) as the product was only weakly dextrorotatory. Repeated attempts to determine the presence of 2,4-cholestadiene by maleic anhydride condensa-

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tion resulted in the formation of an acidic product but this was probably a mixture of acids from maleic anhydride addition compounds, the separation of which was not accomplished. It was thus indicated that in addition to cholesterilene, at least two dextrorotatory compounds are produced by the heat treatment of cholesterol with zinc dust.

Cholesterilene is only slightly adsorbed from petroleum ether solution on activated alumina and hence colored and other adsorbable impurities (including cholesterol) may be easily removed by this means. During the course of the purification of cholesterilene by repeated recrystallization from ether-methanol and from ethyl alcohol on long standing, it was observed that the crystals changed in crystalline structure and became opaque. An alcohol insoluble fraction was isolated by extraction of these opaque crystals with hot alcohol and also by adsorption on activated alumina from petroleum ether solution followed by extraction with ether. This alcohol insoluble solid was also isolated in variable quantities from cholesterilene which had been heated in the absence of a solvent, by adsorption on activated alumina from petroleum ether solution, extraction of the alumina with ether, and repeated reprecipitation of the extract from ether solution by the addition of methonal to yield a white amorphous solid (m.p. 165° (bloc Maquenne), $(\alpha)^{27}{}_{D}+24.3°$ (c, 3.23 in CCl_4)). This may be a peroxide of cholesterilene since the analysis indicated the empirical formula $C_{27}H_{44}O_2$ and since it liberated iodine from potassium iodide. In general, the sample of cholesterilene of higher negative specific rotation were more resistant to heat since these samples decreased less in laevorotation and less of the dextrorotatory solid was produced. The specific rotation of samples of cholesterilene occasionally becomes less negative even on recrystallization and samples of cholesterilene prepared by the zinc dust method were found more susceptible to marked drops in negative rotation than cholesterilene prepared by the copper sulfate method. Thus a highly laevorotatory sample of cholesterilene prepared by the copper sulfate method has been observed to drop a few degrees whereas one sample of cholesterilene prepared by the zinc dust method dropped as much as 26° in a single recrystallization. This greatly hinders the purification of samples of cholesterilene since it was not found controllable.

EXPERIMENTAL

PREPARATION OF CHOLESTERILENE BY THE COPPER SULFATE METHOD

The reaction product obtained by heating an intimate mixture of equal weights of dry cholesterol and powdered anhydrous copper sulfate in an Erlenmeyer flask placed in an oil bath maintained at 175° or 200° was extracted with alcohol, treated with decolorizing carbon, filtered, and allowed to cool. The crude cholesterilene thus obtained was repeatedly recrystallized from various solvents, chiefly ether-methanol and ethyl alcohol, to yield purified cholesterilene. Equal weights of dry cholesterol and powdered anhydrous copper sulfate in an Erlenmeyer flask provided with an air condenser were also refluxed in xylene or toluene (4 cc. per gm. of cholesterol) on a hot plate and the copper sulfate was separated by decantation and washed with ether. The decanted solution and the ether washings were combined and the solvent was removed by distillation under reduced pressure. The residue was dissolved in hot alcohol, treated with decolorizing carbon, filtered, and cooled. The crude cholesterilene in

petroleum ether solution was passed through a column of activated alumina (Alorco, 50 to 200 mesh, freshly heated at 250° for 2 hours) and repeatedly recrystallized from ether-methanol and alcohol to yield purified cholesterilene. The constants observed for the products obtained are presented in table 1.

TABLE 1. *Physical constants of cholesterilene*

Reaction temperature in degrees C	Time in hrs.	Crude product				Purified product			
		M.P. in °C	Optical rotation $(\alpha)_D$	c	°C	M.P. in °C	Optical rotation $(\alpha)_D$	c	°C
200*	0.33	75-77	− 85.3°	3.12	25	79.5-80	−104.91°	3.00	25
175	0.75	76-77.5	− 92.5°	3.46	25	79.5-80	−106.2°	3.20	26
140 (in xylene)	7.00	77-78.5	−101.2°	3.08	23	79.5-80	−118.6°	3.16	24
111 (in toluene)	30.00	77-78	−103°	3.17	24	79.5-80	−117.5°	3.31	21

* The constants observed for the purified product obtained under these conditions are included as previously found (2).

A convenient method for the preparation of practically pure cholesterilene in good yield by the dehydration of cholesterol by means of copper sulfate in xylene was thus developed. From 25 gm. of cholesterol, 16 gm. of cholesterilene ($(\alpha)^{23}_D - 101.2°$) were obtained. This was dissolved in 100 cc. of petroleum ether and passed through an 18 x 380 mm. column of activated alumina. The column was eluted with 150 cc. of petroleum ether and the combined filtrates were concentrated. The residue was crystallized once from alcohol to give 14.8 gm. of colorless cholesterilene ($(\alpha)^{21}_D - 104.4°$) which was then recrystallized until the specific rotation was − 118.6°.

TREATMENT OF CHOLESTEROL WITH ZINC DUST

In a 50 cc. distilling flask a mixture of 13 gm. of dry cholesterol and 100 gm. of zinc dust (Baker, 82.6 per cent) was heated at 260-5° for 45 minutes under the reduced pressure of a water pump. The reaction product was extracted with a total of 200 cc. of petroleum ether (b.p. 68-77°) and the extract was passed through an 18 x 380 mm. column of activated alumina to remove the color and unchanged cholesterol. The column was eluted with 150 cc. of petroleum ether and the filtrates were combined. The solvent was removed by distillation under reduced pressure to yield 7.7 gm. of a colorless oil ($(\alpha)^{22}_D + 5.55°$ (c, 3.24 in CCl_4)). This oil was extracted several times with hot alcohol and the alcohol soluble fraction was removed by decantation. The alcohol insoluble fraction was repeatedly reprecipitated from ether solution with methanol to yield 2.2 gm. of a white amorphous solid which melted at 112° (bloc Maquenne) and $(\alpha)^{24}_D$ was + 70.6° (c, 3.06 in CCl_4). This product was unaffected by refluxing for 10 hours in 300 cc. of alcohol containing 4 cc. of concentrated hydrochloric acid.

The alcohol soluble extract was refluxed with 4 cc. of concentrated hydrochloric acid for 10 hours, treated with decolorizing carbon, and cooled to crystallize the product (cholesterilene) in needles (3.2 gm.) (m.p. 75-77°, $(\alpha)^{22}_D$—89.36° (c, 3.10 in CCl_4)) which on repeated re-

crystallizations melted at 76-77.5° and $(\alpha)^{24}_{D}$ was —92.6° (c, 2.92 in CCl_4). The highest negative specific rotation observed for a sample of cholesterilene (still impure) prepared by the zinc dust method was — 96.2°. From the alcohol soluble fraction were isolated needles ($(\alpha)^{23}_{D}$+21.6° (c, 2.92 in CCl_4)) which when refluxed with 300 cc. of alcohol containing 4 cc. of concentrated hydrochloric acid yielded cholesterilene in needles (m.p. 75-76.5°, $(\alpha)^{25}_{D}$— 87.0° (c, 3.63 in CCl_4)).

The alcohol soluble fraction was treated with maleic anhydride in benzene, toluene, and xylene solution followed by saponification according to the method of Stavely and Bergmann (5) but a definite melting point for the acidic product obtained was not observed and repeated attempts to purify the product indicated that it was a mixture of acids from maleic anhydride addition compounds. The maleic anhydride addition compound, m.p. 265° with decomposition, was obtained by the same procedure from 2,4-cholestadiene which was prepared by a modification of the method of Stavely and Bergmann (5) and of Skau and Bergmann (4) as follows: In a 125 cc. Erlenmeyer flask provided with an air condenser, a mixture of 5 gm. of dry cholesterol, 5 gm. of activated alumina (200 mesh), and 30 cc. of xylene was refluxed for 8 hours in an oil bath. Petroleum ether was added to the reaction product and the solution was passed through an 18 x 210 mm. column of activated alumina (50 to 200 mesh) which was then eluted with petroluem ether. The combined filtrates were concentrated and the residue was crystallized from alcohol to yield 0.9 gm. of 2,4-cholestadiene, m.p. 66-7°, $(\alpha)^{21}_{D}$+ 142°, which on recrystallization from ether-methanol melted at 67-8° and $(\alpha)^{24}_{D}$ was + 158.6°.

EFFECT OF HEAT ON CHOLESTERILENE

A sample of cholesterilene prepared by the copper sulfate method with a specific rotation of —103.8° was found to have a specific rotation of — 101.2° after heat treatment at 100° for 14 days. In a cork stoppered vial 0.545 gm. of cholesterilene prepared by the copper sulfate method with a specific rotation of —84.7° was heated at 100° for 9 days and the specific rotation was found to have become considerably less negative ($(\alpha)^{26}_{D}$— 2.88° (c, 5.45 in CCl_4)). The contents of the vial were dissolved in petroleum ether and passed through a column of activated alumina. The alumina was extracted with ether to give 0.229 gm. of a pale yellow oil ($(\alpha)^{25}_{D}$+ 24.0° (c, 2.29 in CCl_4)) which when repeatedly recrystallized from ether solution with methanol yielded a white amorphous solid (m.p. 165° (bloc Maquenne), $(\alpha)^{27}_{D}$+ 24.3° (c, 3.23 in CCl_4)), the analysis of which indicated the empirical formula $C_{27}H_{44}O_2$ and which liberated iodine from potassium iodide and gave molecular weight values of 355, 361, and 359 (camphor) with the evolution of a gas.

Anal.: Calc'd for $C_{27}H_{44}O_2$: C, 80.92; H, 11.08.
Found: C, 81.15, 80.67, 81.00; H, 11.10, 11.08, 11.21.

Likewise a sample of cholesterilene prepared by the zinc dust method with a specific rotation of — 83.2° when heated at 100° for 24 hours gave a brown glassy solid with a specific rotation of — 7.5° from which the above dextrorotatory solid was obtained. The use of adsorption on activated alumina is also of value in the purification of cholesterilene. Thus the ether extract (yellow oil) of an alumina column through which had

been passed a petroleum ether solution of the reaction product obtained by heating cholesterol with copper sulfate at 200° for 20 minutes had a low specific rotation ($(\alpha)^{23}{}_D + 0.85°$ (c, 1.61 in CCl_4)) whereas the cholesterilene from the filtrate of the column had a specific rotation of — 82.1°.

SUMMARY

In the preparation of cholesterilene by the copper sulfate method, the conditions employed were found to be of more importance than the subsequent purification of the product obtained. Although cholesterilene (3,5-cholestadiene) of the highest attained purity is obtained by the pyrolytic decomposition of cholesteryl methyl xanthogenate and by the action of alcoholic hydrochloric acid on allo- or epiallocholesterol, practically pure cholesterilene is most conveniently prepared in good yield by the dehydration of cholesterol with anhydrous copper sulfate.

Heat treatment of cholesterol with zinc dust produces cholesterilene and two dextrorotatory products, one of which was indicated to be 2,4-cholestadiene. 2,4-Cholestadiene was prepared by the dehydration of cholesterol with alumina in xylene. Heat treatment of cholesterilene was found to produce a substance with an empirical formula of $C_{27}H_{44}O_2$ which may be a peroxide of cholesterilene since it liberated iodine from potassium iodide.

REFERENCES

1. Eck, J. C., and R. L. Van Peursem
1939. The dehydration of cholesterol. Iowa State Coll. J. Sci., 113:115-128.

2. Eck, J. C., R. L. Van Peursem, and E. W. Hollingsworth
1939. The preparation of 3,5- and 4,6-cholestadienes and a study of cholesteriilene and of "7-dehydrocholestene isomer." J. Am. Chem. Soc., 61:171-174.

3. Fantl, P.
1926. Zinkstaubdestillation des Cholesterins. Monatsh., 47:251-258.

4. Skau, H. E. and W. Bergmann
1938. The chemistry of unsaturated steroids. IV. The preparation and photochemical oxidation of 2,4-cholestadiene. J. Org. Chem., 3:166-174.

5. Stavely, H. E., and W. Bergmann
1937. The chemistry of unsaturated steroids. II. The preparation and properties of 2,4-cholestadiene. J. Org. Chem., 1:575-579.

NEW SPECIES AND RECORDS OF SIPHONAPTERA FROM MEXICO

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The following paper is based upon a small collection of fleas taken by Mr. Harry Hoogstraal at Nuevo Leon in Mexico. Of the eight species represented three are semi-cosmopolitan, three are known from Western United States, and two are described as new. The types and other specimens are deposited in the collection of Iowa State College, Ames, Iowa.

Family HECTOPSYLLIDAE

Echidnophaga gallinacea Westwood

1875 *Sarcopsyllus gallinaceus* Westwood, Ent. Mo. Mag. 11: 246.
1906 *Echidnophaga gallinacea* Jordan and Rothschild, Liverpool Univ., Thompson Yates and Johnson Lab. Rep. 7 (n.s.) p. 52.

Record: Mexico—Ojo de Agua, Craleano, Nuevo Leon, August 12, 1938, on "*Citellus v. couchi,*" three females; same locality, July 13, 1938, on "pocket gopher," female.

Family PULICIDAE

Pulex irritans Linnaeus

1758 *Pulex irritans* Linnaeus, Systema Naturae, p. 614.

Record: Mexico—Ojo de Agua, Craleano, Nuevo Leon, August 10, 1938, on "dog," female, four males; same locality, August 13, 1938, on "*Citellus v. couchi,*" male.

Ctenocephalides canis Curtis

1826 *Pulex canis* Curtis, British Entomology, Vol. III, No. 114, Figs. A-E, 8.
1930 *Ctenocephalides canis* Stiles and Collins, United States Public Health Ser. Rep. 45: 1308.

Record: Mexico—Ojo de Aqua, Craleano, Nuevo Leon, August 10, 11, 1938, on "dog," numerous specimens.

Family DOLICHOPSYLLIDAE

Diamanus montanus Baker

1895 *Pulex montanus* Baker, Can. Ent. 27: 132 (Fort Collins, Colorado, on "large mountain gray squirrel (*Sciurus aberti?*)").
1904 *Ceratophyllus acutus* Baker, Invertebrata Pacifica 1: 40 (Stanford University, California, on *Spermophilus*).
1904 *Ceratophyllus montanus* Baker, Proc. United States Nat. Mus. 27: 388, Pl. XXII, figs. 7-8, Pl. XXIII, figs. 1-5 (southern Colorado and Arizona on rock squirrel).

1905 *Ceratophyllus montanus* Baker, Proc. United States Nat. Mus. 29:135.
1929. *Ceratophyllus montanus* Jordan, Nov. Zoo. 35:31.
1933 *Diamanus montanus* Jordan, Nov. Zoo. 39:73.

Record: Mexico—Ojo de Aqua, Craleano, Nuevo Leon, August 11, 1938, on "dog," female; same locality, August 10-15, 1938, on *"Citellus v. couchi,"* numerous specimens.

Since this species is widely distributed throughout southwestern United States where it parasitizes the ground squirrel in particular, its occurrence in northern Mexico is not surprising.

Pleochaetis sibynus Jordan

Plate I, Fig. 3

1925 *Ceratophyllus sibynus* Jordan, Nov. Zoo. 32:110, fig. 42 (Paradise, Arizona, on "skunk").
1933 *Pleochaetis sibynus* Jordan, Nov. Zoo. 39:77.

Male. Frontal tubercle distinct and acuminate. Preantennal region of head armed with two rows of bristles; upper row consisting of seven bristles of various sizes, lower row consisting of three very long bristles. Genal process acuminate. Labial palpus reaching almost to the apex of the fore coxa. Second antennal segment armed with a number of very short bristles. A series of small setae along posterior margin of antennal groove. Postantennal region of head armed with three rows of bristles; first row consisting of two or three, second row of four or five, third row of five or six bristles. Pronotum armed with a single row of alternating long and short bristles and a ctenidium of 19 or 20 spines. Meso- and metanotum each armed with a posterior row of long bristles anterior to which are two or three rows of much shorter bristles. Each abdominal tergite armed with two rows of bristles, the anterior tergites further armed with one or two short stout dorsal teeth on a side. Fifth tarsal segment of each leg with five pairs of lateral plantar bristles of which the basal pair is strongly displaced towards the median line. *Modified segments.* Movable finger variable in shape; of the five specimens at hand, three correspond with the figure published by Jordan in his original description while the other two show marked similarity to Jordan's *P. equatoris*[1] in the structure of the movable finger as is demonstrated by Plate I, fig. 3. Posterior margin of movable finger armed with three long bristles and a stout spiniform. Process of clasper more or less triangular in shape, its apex armed with three small bristles. Manubrium short, blunt apically. Penis broad and blade-like, terminating in a curved process; spring long but not completing more than one turn. Sternite VIII not expanded apically; bearing a number of short bristles along the posterior margin and two more robust ones apically.

Records: Mexico—Ojo de Aqua, Craleano, Nuevo Leon, July 20, 24, and August 10, 1938, on *"Peromyscus,"* four males; Cerro Potosi, Nuevo Leon, 12,500 ft., July 28, 1938, on *"Microtus,"* male.

[1] Nov. Zoo., 38:344, Fig. 63, 1933 (Ecuador, on *Sigmodon* sp.).

Foxella mexicana, n. sp.

Plate I, Figs. 1, 4

Female. Frontal tubercle minute. Preantennal region of head armed with two rows of bristles; both upper and lower rows armed with about six bristles. Genal process acuminate. Labial palpus reaching to the apex of the fore coxa. Second antennal segment armed with about ten long bristles which extend beyond the apex of the third antennal segment. A series of small setae along the posterior margin of the antennal groove. Postantennal region of head armed with a single bristle in addition to the marginal row of six or seven (Plate I, fig. 1). Pronotum armed with a single row of alternating long and short bristles and a ctenidium of 24 or 25 spines. Mesonotum armed with two distinct rows of stout bristles anterior to which are numerous much weaker ones. Metanotum armed with only two rows of bristles. Each abdominal tergite armed with a row of exceedingly long bristles anterior to which are two irregular rows of much shorter ones. Fifth tarsal segment of each leg armed with five pairs of lateral plantar bristles. Sternite VII with a sinus ventally. Head of receptaculum seminis round, about as wide as long; tail not longer than the head. For further details concerning the structure of the female genitalia see Plate I, fig. 4.

Type material. Female holotype and female paratype collected July 13, 1938, from "pocket gopher" at Cerro Potosi, Nuevo Leon, Mexico.

This new species may be readily separated from *Foxella ignota* (Baker) by the distinctive chaetotaxy of the head and by the genitalia.

Malareus jordani, n. sp.

Plate I, Fig. 2

Male. Frontal tubercle prominent, acuminate. Preantennal region of head armed with an ocular row consisting of three bristles above which is a series of three shorter bristles near the antennal groove. Genal process acuminate. Labial palpus extending beyond the apex of the fore coxa. Second antennal segment with several short bristles which barely reach to middle of third antennal segment. A series of small setae along posterior margin of antennal groove. Postantennal region of head armed with a marginal row of bristles and a long stout bristle near the second antennal segment above which is a much shorter one. Pronotum armed with a row of alternating long and short bristles and a ctenidium of about 17 spines. Meso- and metanotum each armed with a posterior row of long bristles anterior to which is another row of much shorter bristles. Each abdominal tergite armed with two rows of bristles, the anterior tergites furtherarmed with one or two short stout dorsal teeth on a side. Fifth tarsal segment of each leg armed with five pairs of lateral plantar bristles of which the basal pair is strongly displaced towards the median line. *Modified segments.* Process of clasper broad and lobular armed with four short bristles at the apex. Movable finger broad, concave at both margins, armed with five bristles on the posterior margin of which the basal three are stout spiniforms. Another spiniform, somewhat shorter than the rest, located near the second from the basal spiniform (Plate I, fig. 2). Manubrium blunt distally. Penis broad and heavy, not acuminate distally;

spring very short not completing a single turn. Sternite VIII armed with two bristles located on posterior margin some distance below apex.

Type material. Male holotype collected July 20, 1938, from "*Peromyscus*" at Cerro Potosi, Nuevo Leon, Mexico.

This species closely resembles *M. bitterrootensis* (Dunn and Parker) from Montana and *M. euphorbi* (Rothschild) from British Columbia. From these species it may be readily separated by the details of genitalia (Plate I, fig. 2) as well as other structures.

Family ISCHNOPSYLLIDAE

Sternopsylla texana, C. Fox

1914 *Ischnopsyllus texanus* C. Fox, United States Public Health Ser. Hyg. Lab. Bull. 97: 16, Pl. V, figs. 6-8 (Pecos, Texas, on bat, "*Nyctenomus mexicanus*")

1921 *Sternopsylla texana* Jordan and Rothschild, Ectoparasites 1: 158.

Record: Mexico—Cerro Potosi, Nuevo Leon, August, 1938, on "*Leptonycteris nivalis,*" three females.

PLATE I

EXPLANATION OF PLATE I

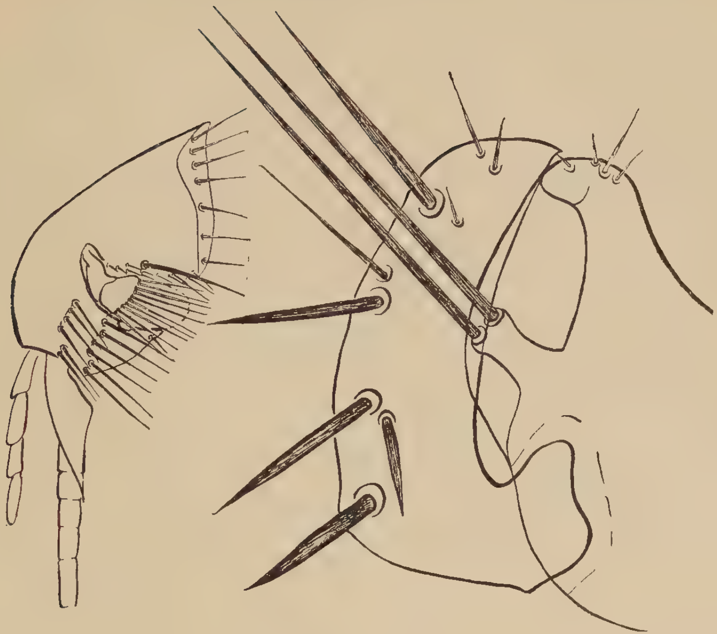
FIG. 1. *Foxella mexicana,* n. sp., female, head.

FIG. 2. *Malareus jordani,* n. sp., male, movable finger and process of clasper.

FIG. 3. *Pleochaetis sibynus* (Jordan), male, movable finger and process of clasper.

FIG. 4. *Foxella mexicana,* n. sp. female, receptaculum seminis and sternite VII.

PLATE I



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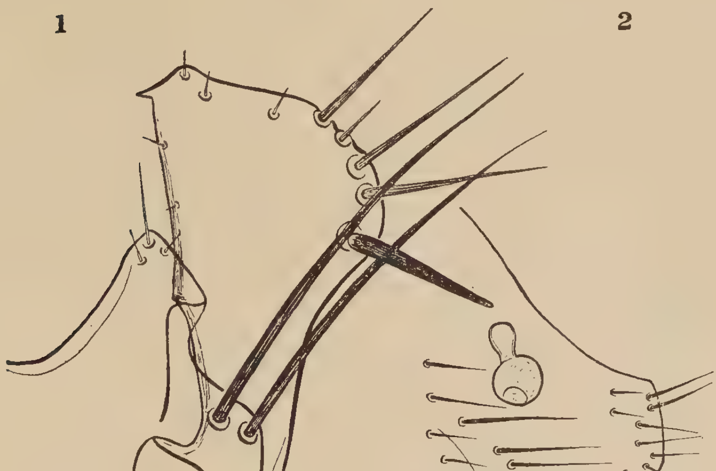

3 4

NOTES ON TYPHA ANGUSTIFOLIA L. IN IOWA[1]

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Received June 21, 1939

While making a survey in Clay and Palo Alto Counties of the plant cover sheltering waterfowl (Plate I), it was observed that the cat-tail population was not uniform. In shallow lakes and marshes in the vicinity of Ruthven during the past five years (1933 to 1938), plants of the type described as *Typha angustifolia* L. have been more widespread than *T. latifolia* L. Dr. W. A. Anderson has located a colony of *Typha angustifolia* from which specimens have been collected in the vicinity of Lake Okoboji in Dickinson County, and Dr. H. S. Conard reported to the writer a collection from Rush Lake in Osceola County. The name only appears for the first time in Conard's Key to Plants of Iowa (Grinnell Flora 5th ed. 1939), though no written discussion of *T. angustifolia* in Iowa has been noted. The occurrence of *T. latifolia* has been reported in several adjoining states; in Missouri (7), local in Saline County, in the shallow water of a spring-fed lake; in Wisconsin (2), occurring in marshes in the vicinity of Madison; in central Kansas (3), in salt marshes; in Illinois (8), abundant locally in marshes surrounding Stony Island, rare at other points in the vicinity of Chicago; locally in South Dakota (6), in water which is more or less alkaline.

T. angustifolia (Plate IV) is less prominent in Clay and Palo Alto Counties than its variety *T. angustifolia* L. var. *elongata* (Dudley) Wiegand (Plate II, fig. 2) which occurs in dense colonies and borders on the north side of Mud Lake, along the south and west shores of Lost Island Lake and its outlet (Plate III), around the west bay and shore of Trumbull Lake, in colonies in Elk and Virgin Lakes and in large areas particularly along the east third of Round Lake. In the summer of 1938 a large colony of *T. angustifolia* var. *elongata* was located in New Lake, an oxbow lake in Woodbury County, about one-half mile west of the town of Salix.

Table 1 compares the chief characters that distinguish the main types of Typha thus far observed. However, some intermediate types which appear to be hybrids cannot yet be included because of insufficient data.

In the shallow lakes of Clay and Palo Alto Counties the cat-tail phase of the emergent flora advances or recedes with the rise or fall of the water level which varies in depth from a few inches to two or three feet around the cat-tails. In 1935 and 1936 some stands were left by the receding water on mud flats for a time. The position of the colonies is affected temporarily through cutting by muskrats which assist in the cycle of succession.

In 1937 many of the cat-tails were attacked by micro-organisms which caused rotting of the rootstock after which the plants collapsed and sank under the water. The stands of the narrow-leaved cat-tails during the five years observed in such shallow lakes as Round, Mud, and Trumbull maintained themselves largely in vigorous condition and covered a

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[1]Journal Paper No. J647 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 366.

TABLE 1. *Comparative descriptive characteristics of Typha*

Species	Vegetative structures			Inflorescence			
	Height	Stem	Leaves	Size of spike in fruit	Color	Pollen	Bractlets
Typha angustifolia	1-1.5 m.	Slender	Convex on back, lower leaves 3-7 mm. wide	8-13 cm. l. 10-17 mm. in diam.	Cinnamon with black markings	Single	Pistillate fl. with bractlets
Typha angustifolia var. elongata	2-3.5 m.	Slender	Flat, lower leaves 9-15 mm. wide	15-25 cm. l. 20-23 mm. in crown	Cinnamon with black markings	Single	Pistillate fl. with bractlets
Typha latifolia	1-2 m.	Stout	Flat, lower leaves 9-15 mm. wide	8-15 cm. l. at least 25 mm. in diam.	Dark brown with black markings	In 4's	Pistillate fl. without bractlets
Typha (intermediate types)	In general aspect like T. angustifolia or its variety					In 4's	

greater area than *T. latifolia* (Plate II, fig. 1) which usually occurs in shallower water, or in the fresh water of hilltop springs known as hanging bogs. Associated with *T. angustifolia* and its variety were some narrow-leaved forms in which the gross aspects of the spike resembled the narrow-leaved type. The pollen grains, however, occur in fours, which structure is characteristic of *T. latifolia.* Other forms in stature intermediate between the two species were seen. These structural variations seem indicative of hybridity.

In the herbarium of Iowa State College is a folder of plants designated Typha. These specimens, in their macroscopic morphology are typical of *T. angustifolia* or its variety, but microscopic inspection shows that they have tetrad pollen grain. This rather robust type which, judging from herbarium specimens, is more or less widespread inland, appears to be a product of the inland environment where it exists with its probable parent species. The relative distribution of these types inland will be revealed, perhaps, by more extended field work and laboratory study.

The water of the lakes mentioned has a pH of 7.5 to 8.5 in the neighborhood of the cat-tails, though higher readings have been made elsewhere. The bottom of the lake is heavily silted (4) through long action of erosional forces operating on the adjacent land. The soil is highly calcareous through the accumulation of plant residues for long periods, and by leaching of salts from the upland (1).

That *T. angustifolia* and its variety have not been more frequently collected is perhaps accounted for by their occurrence in the deeper water of marshes and lakes than *T. latifolia* and by the prevailing idea that *T. angustifolia* is a plant restricted to coastal salt marshes. According to records, the narrow-leaved cat-tail is recurrent inland in saline areas or in the water of alkaline lakes, the soils of which are highly calcareous and where there is poor drainage.

Svenson (11), in a survey of effects of post-pleistocene submergence, finds that the botanical evidence of oceanic submergence offered by early writers as an explanation for the presence of halophytic plants is of little value in accounting for their distribution inland. He states that inland areas with impeded drainage where the underlying rocks are calcareous afford a favorable habitat. In Clay (9) and Palo Alto (10) Counties the underlying rock lies so far below the surface that it does not affect the character of the soils and the waters of the shallow lakes and ponds are derived from surface runoff rather than from subterranean sources. However, the poor drainage resulting in the retention of accumulated vegetative residues which decompose into peat and muck, as well as the products of silting and leaching from higher ground contributes, to a highly calcareous substratum. In these areas such dwellers in brackish costal marshes as the narrow-leaved cat-tail are able to persist inland. Metcalf (5) in researches on North Dakota lakes has determined the total concentration of salts in 75 lakes from which the vegetation is listed. From these data it appears that some of the waters of higher concentrations support only plants that range in America from fresh through brackish, to salt water, such as *Potamogeton pectinatus* and *Ruppia maritima.* It seems probable, therefore, that high concentrations of soil solutions may have a bearing on the tolerance of inland plants, which are also identified with coastal salt marshes.

The foregoing discussion reports for the first time *Typha angustifolia*

and its variety *elongata* as relatively abundant locally in northern Iowa. Associated with the narrow-leaved types are forms intermediate between *T. angustifolia* or its variety and *T. latifolia*. The inland environment is compared with the coastal marshes. Iowa collections upon which this discussion is based and some others are filed in the herbarium of Iowa State College.

LITERATURE CITED

1. Brown, P. E.
1936. Soils of Iowa. Iowa Agr. Exp. Sta. Spec. Rept. No. 3:146.

2. Fassett, Norman C.
1930. Preliminary reports on the flora of Wisconsin. Typhaceae. Trans. Wis. Acad. Sci. Arts and Let. 25:183-184.

3. Gates, Frank C.
1933. Wild flowers in Kansas. Report of the Kansas State Board of Agr. Topeka, Kansas.

4. Report of State Highway Commission
1916. Iowa Lakes and Lakebeds.

5. Metcalf, Franklin P.
1931. Wild duck foods of North Dakota lakes. U.S.D.A. Tech. Bul. 221.

6. Over, Willliam H.
1932. Flora of South Dakota. Univ. of S.D. Vermilion.

7. Palmer E. J., and J. A. Steyermark
1935. An annotated catalogue of the flowering plants of Missouri. Ann. Mo. Bot Gard. 22:375-758.

8. Pepoon, H. S.
1927. An annotated flora of the Chicago area. Chicago Academy of Sciences. Nat. Hist. Surv. Bul. 8.

9. Stevenson, W. H., P. E. Brown, with the assistance of G. E. Corson, T. H. Benton and M. E. Olson
1919. Clay County soils. Soil Surv. of Iowa. Reports No. 12.

10. Stevenson, W. H., P. E. Brown, with the assistance of A. M. O'Neal, Jr., L. W. Forman and W. G. Baker
1922. Palo Alto County soils. Soil surv. of Iowa. Report No. 22.

11. Svenson, H. K.
1927. Effects of post-pleistocene marine submergence in eastern North America. Rhodora, 29:41-48, 57-72, 87-93, 105-114.

PLATE I.

Waterfowl feeding on duckweed in a narrow-leaved cat-tail marsh. Outlet of Lost Island Lake, Clay County.

PLATE I.

PLATE II.

FIG. 1. A zone of *Typha latifolia* L. in shallow water in Dewey's Pasture, Clay County.

FIG. 2. A colony of *Typha angustifolia* L. var. *elongata* (Dudley) Wiegand in Mud Lake, Dewey's Pasture, Clay County.

PLATE II.

PLATE III.

Typha angustifolia L. var. *elongata* (Dudley) Wiegand (cat-tails) in Barringer's Slough, Clay County.

PLATE III.

PLATE IV.

The narrow-leaved cat-tail (*Typha angustifolia* L.)—a service and shelter plant for waterfowl, growing in Round Lake, Clay County.

PLATE IV.

THE EFFECT OF TREATED FATS ON VITAMIN A POTENCY[1]

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Fridericia (1) has reported that hydrogenated whale oil, when mixed with fat containing vitamin A, causes destruction of this vitamin. Ordinary whale oil, according to Drummond (2) and Fridericia (1), contains this dietary factor; whereas, hydrogenated whale oil does not contain this vitamin. It is therefore evident that hydrogenation causes some chemical change in the oil resulting in the formation of certain products that destroy vitamin A. Fridericia also states that heated lard causes destruction of vitamin A, when mixed with fat containing this vitamin; whereas, unheated lard does not have this effect. He reports, furthermore, that certain hydrogenated and nonhydrogenated vegetable fats, when mixed with fat containing vitamin A, do not cause destruction of this vitamin. Powick (3) found that vitamin A in butterfat was destroyed when fed with rancid fats, and Mattill (4) also showed that both vitamins A and E were destroyed by rancid fats. Whipple (5) found that vitamin A in cod liver oil is destroyed as rancidity develops in the oil. For several years Nelson, Nelson, and Lowe (6) have published results on the effect of heated fats on vitamin A. Recently Lease, Lease, Weber, and Steenbock (7) have published work on the effect of rancid and heated fats on carotene and vitamin A. They cite evidence to show that fats which had been heated at such temperatures as are frequently used in cooking destroyed vitamin A to some extent.

Since heated lard causes destruction of vitamin A when mixed with fat containing this vitamin, it seemed important to ascertain whether or not vegetable fats act similarly. Fridericia (1) did not study the action of either heated hydrogenated vegetable fats or heated nonhydrogenated vegetable fats upon vitamin A. Experiments were therefore instituted in order to answer the following questions: First, what effect do heated and unheated fats have on vitamin A activity of butterfat when mixed with the latter fat? Secondly, from a practical standpoint, will baking with lard or other fats cause destruction of vitamin A in the baked product? No distinction in this work has been made between vitamin A and carotene; consequently the observed effect is that of treated fats on vitamin A potency or activity.

EXPERIMENTAL

All of the experiments were performed on rats weighing from 45 to 55 gms. when placed on the various rations; they were obtained from the stock colony maintained by the Chemistry Department, Iowa State College. They were housed in wooden frame cages, the sides of which were composed of hardware cloth; galvanized pans served as bottoms and wood

[1] Journal Paper No. J-661 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 328.

shavings were employed as litter. Six rats, three males and three females, were placed in each lot; they were weighed weekly and cared for daily. The following basal ration was employed in the tests: casein 200 gms., salts 50 gms., yeast 100 gms., and dextrin 450 gms. The casein was prepared by washing the commercial product daily with 0.15 per cent acetic acid until free of vitamin A. The salt mixture was essentially the one employed by McCollum and Davis (8) and designated by them, salt mixture 185. It contained additional small quantities of copper and manganese salts together with a small amount of potassium iodide. The yeast was a dried product obtained from Standard Brands, Inc. Dextrin was made by treating starch with 0.37 per cent citric acid solution and autoclaving at 15 pounds pressure for three hours.

The data in this paper will be considered under two heads: First, the effect of individual fats, heated and unheated, on the vitamin A activity of butterfat when mixed with the latter fat; and, secondly, the effect of bakng on the vitamin A activity of butter or egg yolk in cookies containing different fats.

THE EFFECT OF HEATED AND UNHEATED FATS ON VITAMIN A POTENCY

The following four rations, similar to those employed by Fridericia (1), were used in this study: Ration I consisted of 800 gms. of the basal ration and 200 gms. of butterfat; ration II consisted of 900 gms. of the basal ration plus 100 gms. of butterfat; ration III was composed of 800 gms. of the basal ration plus 100 gms. of butterfat and 100 gms. of the fat investigated; and ration IV consisted of 800 gms. of the basal ration, 100 gms. of butterfat, and 100 gms. of the corresponding heated fat undergoing investigation. The latter had been heated 24 hours in a thin layer (one-eighth inch thick) at 102° C. to 105° C. The butterfat and the fat tested were mixed at temperatures slightly above the melting points of the two fats. The butterfat employed in the studies was prepared by heating butter on a steam plate to the melting point, following which the fat was promptly decanted from the salts, water, and curd. Rations I and II served as controls for all of the fats and oils; they were not repeated for each test, although they were employed a total of three times. Growth of the animals was normal on rations I and II. Rations III and IV were employed for each fat studied.

The results of this series of experiments are given in table 1. The effect of heated fats upon vitamin A activity was made evident by the poor growth of the animals receiving the heated fats and the development of xerophthalmia in the animals; each lot was continued on experiment for at least 12 weeks, or until death intervened. The plus sign under growth signifies that body weight increased steadily, and the negative sign signifies that loss of weight occurred after different lengths of time, and that the animals died. The figures under xerophthalmia designate the numbers of animals showing symptoms of the disease and, since six animals were employed in each lot, the remaining animals in the lots died without displaying any evidence of the malady. The letters N. D. signify that vitamin A activity was not destroyed, and the letter D signifies that destruction of this vitamin occurred. U. V. indicates that the animals were exposed to ultra-violet light for five minutes daily. The term storage refers to animal fats kept under specified conditions for varying lengths of time. The storage animal fats (experiments 14, 15, and 16) were kept in a cool place.

TABLE 1. *The effect of heated and unheated fats on vitamin A activity of butterfat*

Exp. No.	Name of fat in text	Basic fat material	Trade name, description or source	Growth U.H.	Growth H.	Xerophthalmia U.H.	Xerophthalmia H.	Effect U.H.	Effect H.
1.	Veg. fat 1	cottonseed oil	Snowdrift	+	—	0	3	N.D.	D.
2.	Veg. fat 2	cottonseed oil	Crisco	+	—	0	3	N.D.	D.
3.	Veg. fat 3	olive oil	Pompeian	+	—	0	2	N.D.	D.
4.	An. fat 4	lard	commercial	+	—	0	4	N.D.	D.
5.	An. fat 4 U.V.	lard	commercial	+	—	0	5	N.D.	D.
6.	An. fat 5	lard	Wilson B. P. S.	+	—	0	5	N.D.	D.
7.	An. fat 6	lard	Wilson O. K.	+	—	0	4	N.D.	D.
8.	An. fat 7 U.V.	lard	Wilson B. P. S.	+	—	0	5	N.D.	D.
9.	An. fat 8 U.V.	lard	Wilson O. K.	+	—	0	5	N.D.	D.
10.	An. fat 9 U.V.	lard	Wilson B. P. S.	+	—	0	5	N.D.	D.
11.	An. fat 10 U.V.	lard	Wilson O. K.	+	—	0	5	N.D.	D.
12.	An. fat 11 U.V.	hydrogenated lard	Clix	+	—	0	5	N.D.	D.
13.	An. fat 12	lard	R. P. (50 per cent leaf; 50 per cent back)	+	—	0	6	N.D.	D.
14.	An. fat 13	storage lard	Wilson O. K.	+	—	0	2	N.D.	D.
15.	An. fat 14	storage lard	Wilson B. P. S.	+	—	0	5	N.D.	D.
16.	An. fat 15	rancid storage lard	commercial	—	—	4	3	D.	D.
17.	An. fat 16	drip lard	French Oil Machinery Co., Piqua, Ohio	+	—	0	4	N.D.	D.
18.	An. fat 12	lard (105°)	R. P.	+	—	0	2	N.D.	D.
19.	An. fat 12	lard (80°)	R. P.	+	+	0	0	N.D.	N.D.
20.	An. fat 12	lard (67°)	R. P.	+	+	0	0	N.D.	N.D.
21.	An. fat 12	lard (37.5°)	R. P.	+	+	0	0	N.D.	N.D.
22.	Oleo 21	oleomargarine	Hollynut	+	+	0	0	N.D.	N.D.
23.	Oleo 21 S	oleomargarine	Hollynut	+	+	0	0	N.D.	N.D.
24.	Oleo 22	oleomargarine	Bestonut	+	+	0	0	N.D.	N.D.
25.	Oleo 22 S	oleomargarine	Bestonut	+	+	0	0	N.D.	N.D.
26.	Oleo 21	oleomargarine	Hollynut	+	+	0	0	N.D.	N.D.
27.	Oleo 21 S	oleomargarine	Hollynut	+	+	0	0	N.D.	N.D.
28.	Oleo 23 S	oleomargarine (mainly coconut oil)	Gem Nut	+	+	0	0	N.D.	N.D.
29.	Oleo 24 S	oleomargarine (mainly oleo oil and neutral lard)	Premium	+	—	0	2	N.D.	D.
30.	Oleo 25 S	oleomargarine (cottonseed oil)	Virginia Belle	+	—	0	3	N.D.	D.

TABLE 1. (continued)

Exp. No.	Name of fat in text	Basic fat material	Trade name, description or source	Growth U.H.	Growth H.	Xerophthalmia U.H.	Xerophthalmia H.	Effect U.H.	Effect H.
31.	Oleo 26	oleomargarine (coconut oil and cottonseed oil)	Nutola	+	—	0	1	N.D.	D.
32.	Oleo 27	oleomargarine (oleo oil, neutral, cottonseed oil)	Silver Churn	+	—	0	4	N.D.	D.
33.	Oleo 28	oleomargarine (partially hydrogenated cottonseed oil)	Good Luck	+	—	0	4	N.D.	D.
34.	An. fat 29	green lard	Wilson	+	—	0	5	N.D.	D.
35.	An. fat 30	green lard	Swift	+	—	0	3	N.D.	D.
36.	An. fat 31	butterfat	College	+	—	0	4	N.D.	D.
37.	Veg. fat 32	coconut oil	commercial	+	+	0	0	N.D.	N.D.
38.	Veg. fat 33	coconut oil	commercial	+	+	0	0	N.D.	N.D.
39.	Veg. fat 34	coconut oil	commercial	+	+	0	0	N.D.	N.D.
40.	Veg. fat 35	coconut oil	commercial	+	—	0	6	N.D.	D.
41.	Veg. fat 36	coconut oil	commercial	+	—	0	5	N.D.	D.
42.	Veg. fat 37	coconut oil	commercial	+	—	0	3	N.D.	D.
43.	Veg. fat 38	coconut oil	commercial	+	—	0	4	N.D.	D.

Note: R. P. refers to record of performance lard. This lard was obtained from animals used in Project 39: "Swine Performance Record," Iowa Agr. Exp. Sta. Record of performance lard is lard made from equal parts by weight of leaf and back fat. Skins are removed and the fat is ground and rendered in a steam jacketed open kettle at a temperature not to exceed 237° F.

Commercial refers to fats in bulk purchased on the local market.

Coconut oil was purchased locally and the coconut oils in the different experiments were different oils from different sources.

An. fat refers to animal fat, and Veg. fat designates vegetable fat.

U.H. signifies unheated, and H. heated.

O. K. refers to open kettle lard, and B. P. S. to bleached prime steam lard.

S signifies that the fat was decanted from salts, water, and curd.

U. V. signifies that the animals were exposed to ultraviolet light for five minutes daily.

N.D. signifies that vitamin A activity was not destroyed; D signifies that vitamin A activity was destroyed.

Green lard is lard possessing a distinct green color.

Unless otherwise mentioned, all of the heated fats were subjected to a temperature of 102° C. to 105° C.

Open kettle storage lard (experiment 14) and bleached prime steam storage lard (experiment 15) were kept in a cooler at 0° C. to 5° C. for seven months and subsequently in a cool room at a temperature between —6° C. and 15° C. for three months. The rancid commercial storage lard (experiment 16) was kept for 10 months in a cool room at a temperature between —6° C. and 15° C.

EXPERIMENTS ON BAKED PRODUCTS

The experiments were performed to ascertain the extent of vitamin A destruction during baking. Cookies served as a convenient baked product to study. Egg yolk or butterfat served as the source of vitamin A in the cookies. The baking temperature was 170° C. for 11 minutes. The size of the cookies was maintained as nearly constant as possible, and the entire baking process was regulated in such a way that errors were reduced to a minimum. Five batches of egg cookies were prepared; the formulae of the cookies are given in table 2. Egg yolk served as a source of vitamin A in these cookies. Batches I and II were prepared for control purposes.

TABLE 2. *Formulae of cookies containing egg yolk as the sole source of vitamin A*

Batch I		Batch II		Batch III	
White flour	276	White flour	276	White flour	276
Sugar	125	Sugar	125	Sugar	125
Water to roll.		Egg yolk	72	Egg yolk	72
		Water to roll.		Animal fat 5*	110
				Water to roll.	

Batch IV		Batch V	
White flour	276	White flour	276
Sugar	125	Sugar	125
Egg yolk	72	Egg yolk	72
Vegetable fat 2*	110	Animal fat 11*	110
Water to roll.		Water to roll.	

* See table 1 for nature of fats used. All figures in tables 2, 3, 4, and 5 are expressed in grams.

Five lots of rats were placed on rations containing the five different cookies the ingredients of which are given in table 2. The rations had the composition shown in table 3.

TABLE 3. *Composition of rations containing the egg cookies*

Lot I		Lot II		Lot III	
Casein	200	Casein	200	Casein	200
Salts	50	Salts	50	Salts	50
Yeast	100	Yeast	100	Yeast	100
Olive oil	147	Olive oil	147	Cookies Batch III	650
Cookies Batch I	553	Cookies Batch II	640		

Lot IV		Lot V	
Casein	200	Casein	200
Salts	50	Salts	50
Yeast	100	Yeast	100
Cookies Batch IV	650	Cookies Batch V	650

The rats in lot I lost weight, exhibited xerophthalmia, and died in from four to six weeks' time. Rats in lot II grew normally. It is evident that the egg yolk supplied all of the vitamin A in the cookies. Animal fat 5, vegetable fat 2, and animal fat 11 when incorporated in the cookies containing the egg yolk as the sole source of vitamin A did not cause destruction of the vitamin A in the baked product, nor was the vitamin destroyed in the cookies containing egg yolk but no added fat. The experiments were continued for as long as 18 weeks; data on growth and xerophthalmia are given in table 6 (experiment A, lots I to V, inclusive). Five batches of cookies with butter as the sole source of vitamin A were then baked; they had the formulae shown in table 4. The butter employed in the experiments recorded in this paper was purchased from the Dairy Industry Department, Iowa State College.

TABLE 4. *Formulae of cookies containing butter as the sole source of vitamin A*

Batch VI		Batch VII		Batch VIII	
White flour	276	White flour	276	White flour	276
Sugar	125	Sugar	125	Sugar	125
Butter	110	Butter	55	Butter	55
Water to roll.		Animal fat 5*	55	Vegetable fat 2*	55
		Water to roll.		Water to roll.	

Batch IX		Batch X	
White flour	276	White flour	276
Sugar	125	Sugar	125
Butter	55	Animal fat 5*	110
Animal fat 11*	55	Water to roll.	
Water to roll.			

* See table 1 for nature of fats used in these batches of cookies.

Five lots of rats were placed on rations containing the cookies of table 4. The composition of the rations fed to the rats is given in table 5.

TABLE 5. *Composition of rations containing butter cookies*

Lot VI		Lot VII		Lot VIII	
Casein	200	Casein	200	Casein	200
Salts	50	Salts	50	Salts	50
Yeast	100	Yeast	100	Yeast	100
Cookies Batch VI	650	Cookies Batch VII	650	Cookies Batch VIII	650

Lot IX		Lot X	
Casein	200	Casein	200
Salts	50	Salts	50
Yeast	100	Yeast	100
Cookies Batch IX	650	Cookies Batch X	650

The animals in lots VI, VII, IX, and X grew for a short time (one-half to two months), then their weights declined, and finally death occurred. Animals in lot VIII grew normally for a period of 16 weeks, when the experiment was terminated. The results of this experiment are given in table 6 (experiment A, lots VI to X, inclusive). A second series of butter

cookies was tested. These cookies were prepared according to the same method and had the same formulae as the first series of butter cookies (table 4). The diets containing the second series of butter cookies had the composition as given in table 5. The experiments on the second series of butter cookies were begun about three and one-half months after the beginning of the first series of butter cookie experiments; the data are recorded in table 6 (experiment B, lots VI to X, inclusive). The results are not in agreement with those obtained on the first series of butter cookies, experiment A, lots VI to X, inclusive. In the first series of butter cookies only lot VIII, receiving vegetable fat 2, grew normally; whereas, in the second series of butter cookies the animals in lots VIII and IX, receiving vegetable fat 2 and animal fat 11, respectively, exhibited normal curves of growth. The other lots ceased to grow after one-half to three months on the rations. The control group, experiment B, lot I, received the same ration as did the animals in lot I, table 3. The experiments on the egg cookies also were repeated, and the results were the same as on the first series of egg cookies. The data on the second series of egg cookies are given in table 6 (experiment B, lots I to V, inclusive).

The butter cookies remaining after experiment A, table 6, was completed were stored at a temperature of 26° C. for 10 months, incorporated into rations having the composition given in table 5, and fed to the animals.

TABLE 6. *Results obtained on the baked products*

Experiment	Lot I	Lot II	Lot III	Lot IV	Lot V	Lot VI	Lot VII	Lot VIII	Lot IX	Lot X
A	—(2)	+	+	+	+	—(3)	—(6)	+	—(4)	—(4)
B	—(2)	+	+	+	+	—(3)	—(4)	+	+	—(4)
C	—(3)					—(4)	—(3)	+	—(5)	—(4)
D	—(2)					+	+	—(6)	+	—(2)
E	—(2)					+	+	+	+	—(2)

+ and — signs designate rate of growth as compared to normal.

Figures refer to the number of animals exhibiting xerophthalmia. The animals were kept on experiment for at least 12 weeks (in some cases as long as 18 weeks) or until death intervened.

The results are recorded in experiment C, table 6. The rats in lot VIII grew normally for 12 weeks, when the experiment was discontinued; the remaining animals ceased to grow at varying times, ranging from one to three months. A third and fourth series of butter cookies were prepared having the same formulae as series I, table 4, and the rations containing the cookies had the composition given in table 5. The third series of butter cookies was kept at 26° C., and enough of each batch of the cookies was made into rations at one time for a three weeks' supply. The data obtained with these cookies are given in table 6 (experiment D, lots VI to X, inclusive). The fourth series of butter cookies was kept in a cold room (—6° C. to 10° C.). Enough of each ration for a three weeks' supply was made each time, but only a one week's supply of the ration was kept in the laboratory, the remainder being kept in the cold room. The results of this experiment are shown in table 6 (experiment E, lots VI to X, inclusive).

The same fats were employed for the preparation of the third and fourth series of butter cookies.

TABLE 7. *Peroxide content of fats*

Fats	Peroxide value (millimols per 1,000 gms.)
Unheated oleo 21	3.90
Heated oleo 21	62.8
Unheated oleo 23	1.04
Heated oleo 23	67.3
Unheated oleo 24	1.06
Heated oleo 24	64.3
Unheated oleo 25	2.78
Heated oleo 25	66.3

DISCUSSION OF RESULTS

The data in table 1 reveal that most of the heated fats, when mixed with unheated butterfat, cause destruction of vitamin A contained in the latter fat. However, two brands of oleomargarine, designated oleo 21 and oleo 22, which were purchased on the local market, acted differently from the other fats. Our first experiments on both margarines were made on the products as purchased. These margarines when heated and mixed with unheated butterfat did not destroy vitamin A of the butterfat (table 1, experiments 22, 24). It might be well to emphasize at this point that all samples of butterfat employed in the experiments, whose results are given in table 1, were prepared from butter by decanting the fat from water, curd, and salts. Since the two brands of oleomargarine acted differently from the other fats, it was believed that this might be caused by the salts or curd; and that, if these substances were removed, the margarines upon heating might act like the other fats. Consequently, another experiment was performed, in which both margarines were melted and the fat layer decanted; the decanted fats were then heated for 24 hours at 102° C. to 105° C. and mixed with unheated butterfat. It will be observed from table 1, experiments 23 and 25, that vitamin A activity of the butterfat was not destroyed when the margarines so treated were mixed with unheated butterfat. The unheated margarines did not cause destruction of vitamin A activity when mixed with unheated butterfat, as was to be expected. In all cases the fat tested and the butterfat were mixed at a temperature slightly above the melting point of the fats and the mixture added in the proper amount to the ration. In experiments 22, 24, and 26 the margarines were employed as purchased; whereas, in experiments 23, 25, and 27, S signifies that decanted fats were used. It would appear that the two margarines, oleo 21 and oleo 22, act differently from other fats on heating. However, because the results on these margarines were unique, it was deemed advisable to repeat the experiments on decanted and nondecanted oleo 21. It will be observed, table 1, experiments 26 and 27, that the results of the second test on oleo 21, heated and unheated, were the same as those obtained on the first sample. Oleo 22 was not tested again, because it was not available on the local market.

The two oleomargarines acted differently from most of the other fats, for example, animal fat 31, as shown by experiment number 36, table 1. Animal fat 31, after melting and decanting, was heated for 24 hours at

102° C. to 105° C. in a thin layer (one-eighth inch thick) and mixed with unheated butterfat; and it will be observed from the data that vitamin A in the unheated butterfat was destroyed. In view of this difference between certain margarines and certain animal fats, it was decided to test some more margarines. Dr. W. Lee Lewis of the Institute of American Meat Packers supplied us with three different oleomargarines. These margarines are called oleo 23, oleo 24, and oleo 25. Oleo 23 was made principally from coconut oil, oleo 24 was manufactured from fat of which approximately 75 per cent was animal fat (made up of oleo oil and neutral lard) and 25 per cent was cottonseed oil, and oleo 25 was prepared entirely from cottonseed oil. The results are given in table 1, experiments numbers 28, 29, and 30. Xerophthalmia or death resulted in the rats on heated oleo 24 and oleo 25, so that none of the animals fed these margarines survived at the end of nine weeks. All but one of the rats were dead on heated oleo 25 at the end of six weeks, and the remaining animal died at the end of nine weeks. Three of the rats on heated oleo 24 were killed because of middle ear infection, two animals died of xerophthalmia, and the remaining one died without evidence of this disease. One of the animals on heated oleo 23 was killed because of otitis media, but the remainder, at the end of 12 weeks, were in good physical condition. This experiment seems to indicate that margarines composed of coconut oil act differently on heating than margarines composed of certain other fats. All three margarines were decanted after melting, in order to eliminate salts, curd, and water; the tests were performed on the decanted fats.

Naturally, the next problem was to explain the action of the margarine prepared from coconut oil. The margarine made of coconut oil (experiment 28) and three samples of coconut oil (experiments 37, 38, and 39) acted similarly in that none of them cause destruction of vitamin A in butterfat when heated and mixed with unheated butterfat. However, certain other samples of heated coconut oil caused destruction of vitamin A when mixed with butterfat (experiments 40, 41, 42, 43). It was believed that the differences obtained with the various margarines might be caused by the quantity of peroxide, but the analyses in table 7 do not support this contention. It will be observed from the data in this table that heated oleo 23 contained more peroxide than heated oleo 24 and heated oleo 25, but the vitamin A activity of butterfat was not destroyed by heated oleo 23, but the vitamin activity was destroyed by the other two heated margarines. Furthermore, heated oleo 21 contained almost as much peroxide as heated oleo 24, nevertheless the effects of these two margarines on vitamin A activity were markedly different. More convincing evidence that peroxides are not the only factors responsible for the destruction of vitamin A is shown by the effects of animal fats 29 and 30 on this vitamin. Both of these heated fats caused destruction of vitamin A, even though they contained only 52.53 and 56.21 millimols of peroxide per 1,000 grams of fat, respectively. The data, therefore, strongly suggest that some unknown substances, aside from peroxides, are responsible in whole or in part for the effects produced by heated fats, and that the heated coconut oil margarine either contained less of these substances or else it contained substances of unknown function, possibly of the nature of anticatalyzers, which retarded the reaction. The work of Olcovich and Mattill (9) lends support to some of these conclusions. They say that the commonly measured stability of vitamin A (carotene) in different vegetable foods exposed to heat and light

does not depend upon the carotene itself or on the presence of oxygen, primarily, but is dependent upon the presence of other substances. Furthermore, they state: "Since carotene in the solid state is decolorized and rendered inert, physiologically, by heat in the absence of oxygen, and since in solution it undergoes this change even more rapidly under the influence of heat or ultraviolet radiation, it follows that the resultant achroocarotene is not a product of oxidation but rather of an intramolecular rearrangement, or possibly of polymerization. Inasmuch as hydroquinone delays the transformation of carotene under these conditions, its function as an antioxidant must be extended to include the capacity to prevent the shift in electrons, if such it be, which attends the thermal or photoelectric change of the unstable to the colorless and more stable form of carotene."

Table 1 also reveals another interesting phenomenon; namely, that the exent of vitamin A inactivation produced by heated fats depends upon the temperature to which the fat is subjected. Experiments number 18, 19, 20, and 21 show that record of performance lard heated at or below 80° C. fails to develop the characteristic substances responsible for vitamin A destruction. The question arises whether the same effects of temperature apply to all fats; unfortunately an answer cannot be given from the data at hand. Furthermore, although heated animal fats 5 and 10 inactivated butterfat (experiments 6 and 11, table 1), they did not do so when the ration was mixed daily.

The stored animal fats (experiments 14, 15, and 16) did not act alike. The unheated commercial storage animal fat 15 (purchased on the market) caused destruction of vitamin A when mixed with butterfat; whereas, the other two unheated stored animal fats did not do this. Animal fat 15 was rancid organoleptically while animal fats 13 and 14 were not. It would appear, therefore, that rancidity is in some manner correlated with the capacity of a fat to destroy vitamin A when mixed with butterfat; in other words, from the standpoint of effect upon vitamin A, the development of rancidity within a fat results in transformations analogous to those produced by heating.

It will be observed from the data in table 6 that animal fat 5, vegetable fat 2, and animal fat 11 did not cause destruction of vitamin A in the cookies containing egg yolk. Experiment A reveals that in the first series of butter cookies vegetable fat 2 prevented destruction of vitamin A activity of the butter contained in the cookies; whereas, animal fat 11 and animal fat 5 did not do this. The data also show that vitamin A was destroyed when butter constituted the only fat in the cookies (lot VI, experiment A).

Experiment B shows that vitamin A of butter was not destroyed in the cookies containing either vegetable fat 2 or animal fat 11, but it was destroyed in the cookies containing animal fat 5 and in the cookies containing butter alone. The same batches of animal fat 5 and animal fat 11 were employed in the butter cookies, experiments A and B, table 6; but since experiment B was performed several months after experiment A the animal fat 5 and animal fat 11 employed in experiment B were older samples. The samples of animal fat 5 and animal fat 11 were stored in a cooler at approximately 0° C. Two different samples of vegetable fat 2 were employed in the butter cookies in experiments A and B. The data indicate either that a chemical change had occurred in the animal fat 11 during

storage which so altered it that when incorporated with the cookies vitamin A destruction was prevented; or, what appears more probable, that the conditions involved in the mixing of the ingredients of the cookies and subsequent heating were not absolutely uniform and, therefore, different results were obtained. It is difficult to prepare two batches of baked products exactly alike even from the same mix, and it is just as difficult to know whether or not they are alike. However, the temperature of the ingredients when mixed was 24.5° C. ± 1° C. Mixing time, rolling, and thickness of the mix were kept constant. Baking temperature was constant at 170° C. for 11 minutes and, because extreme care was used to keep conditions constant, it is difficult to explain the different results obtained in experiments A and B.

The cookies remaining after experiment A was completed were stored for 10 months at a temperature of 26° C. and then fed to the animals. The results are given in experiment C, table 6. Even after this long storage period vegetable fat 2 prevented destruction of vitamin A in the cookies. Two new series of butter cookies were prepared carrying different samples of animal fat 5, vegetable fat 2, and animal fat 11 than were employed in the first two series of butter cookies. The results are recorded in table 6 (experiments D and E, lot VI to X, inclusive). Cookies of series D were kept at approximately 26° C., and cookies of series E were stored at a temperature from —6° C. to 10° C. In experiment D vitamin A was destroyed only in the vegetable fat 2 cookies; while in experiment E vitamin A was not destroyed in any of the cookies. It would appear from the results that storage temperature may play some part in the destruction of vitamin A in baked products.

Three samples of coconut oil (experiments 37, 38, 39) on heating failed to destroy vitamin A in butterfat; whereas, four other samples of coconut oil (experiments 40, 41, 42, 43) on heating caused destruction of vitamin A in butterfat. Now it is possible that some coconut oils contain certain antioxidants or substances of unknown function whose presence prevents the oil from developing the property of vitamin A destruction when subjected to heat. Substances that prevent decomposition of fats have been recognized by Anderegg and Nelson (10); it is probable that substances of like function may play an important role in the preservation of vitamin A in the baking process or in the butterfat when mixed with certain heated fats. In an article entitled "Milk Powders as Food II," Anderegg and Nelson state: "During this work a marked difference was observed between the nutritive value of whole milk powder and skimmed milk powder. This difference cannot be attributed solely to a difference in the fat content; for when skimmed milk powder was suitably supplemented so that the resulting diet conformed in chemical composition to one of whole milk powder, on which very good results concerning reproduction were obtained, the outcome on the skimmed milk powder diet was entirely different. When cod liver oil is incorporated in skimmed milk powder diets, as a source of fat soluble vitamins, it undergoes decomposition giving rise to products strongly suggestive of acrolein. Other highly desicated materials also induce this decomposition. Addition of ethyl alcohol, wheat oil, or water to such mixtures exerts a protective action. Skimmed milk powder diets, upon which rats are sterile, were so changed by the addition of water and administering the cod liver oil separately that fourth generation young have now been obtained." The similarity,

from a chemical standpoint, between the work of Anderegg and Nelson and some of the results of this paper are striking.

SUMMARY

1. Most of the heated fats that were studied caused destruction of vitamin A in butterfat.

2. Some heated oleomargarines and some heated coconut oils destroyed vitamin A; whereas, others did not.

3. The data seem to indicate that peroxides in heated fat are not the only factors concerned in the destruction of vitamin A.

4. Heating fat at or below 80° C. did not develop the characteristic change in the fat which causes inactivation of vitamin A.

5. Rancid fats appear to cause destruction of vitamin A.

6. The effects of the addition of fats to baked products on the vitamin A of the products will depend on the nature of the substances used as a source of vitamin A in the baked products, possibly upon the nature of the fats added, and upon certain unknown factors involved in the baking process.

LITERATURE CITED

1. FRIDERICA, L. S.
1924-25. Inactivating action of some fats on vitamin A in other fats. J. Biol. Chem., 62: 471-485.

2. DRUMMOND, J. C.
1919. Researches on the fat soluble accessory substance. I. Observations upon its nature and properties. Biochem J., 13: 81-94.

3. POWICK, WILMER C.
1925. Inactivation of vitamin A by rancid fat. J. Agr. Res., 31: 1017-1026.

4. MATTILL, H. A.
1927. The oxidative destruction of vitamins A and E. J. A. M. A., 89: 1505-1508.

5. WHIPPLE, DOROTHY V.
1936. The destruction of vitamin A by rancid cod liver oil. Oil and Soap, 13: 231-232.

6. NELSON, V. E., P. MABEL NELSON, and BELLE LOWE
1933 to 1938. Annual reports on agricultural research. Agricultural Experiment Station, Iowa State College.

7. LEASE, E. J., JANE G. LEASE, JANET WEBER, and H. STEENBOCK
1938. Destruction of vitamin A by rancid fats. J. Nutrition, 16: 571-583.

8. MCCOLLUM, E. V., and MARGUERITE DAVIS
1914. Observations on the isolation of the substance in butterfat which exerts a stimulating influence on growth. J. Biol. Chem., 19: 245-250.

9. OLCOVICH, H. S., and H. A. MATTILL
1931. The unsaponifiable lipids of lettuce. J. Biol. Chem., 91: 105-117.

10. ANDEREGG, L. T., and V. E. NELSON
1926. Milk powders as food. II. Observations on the existence of vitamin E. J. Ind. and Eng. Chem., 18: 620-627.

THIAMIN EFFECTS IN BACTERIAL METABOLISM

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A review is given in this report of some of the work done in our laboratory during the past year involving the physiological effect of thiamin or vitamin B_1 upon the living cell. Its specific function in the glucose metabolism of living tissue was first reported by R. A. Peters (1) who was able to demonstrate that pyruvic acid in animal tissue arising from the decomposition of carbohydrates cannot be oxidized in the absence of thiamin. Similar results have been obtained by us employing bacterial cells grown in thiamin deficient media (Cf. references 6, 7, 8, 9, 10). The cells used in this work were grown in the basal medium of Tatum, Wood, and Peterson (2) in the absence of any added thiamin. Their ability to oxidize pyruvate under anaerobic conditions was tested in simple Warburg manometers. The results are shown in table 1.

TABLE 1. *The oxidation of pyruvic acid by Propionibacterium*

	Cell suspension		
	Propionibacterium pentosaceum	*Propionibacterium peterssonii*	*Lactobacillus mannitopoeus*
No activator	85	5	23
Two micrograms thiamin added to suspension	480	120	90

Note: Results in mm^3 CO_2 evolved in 3 hours from sodium pyruvate.

The catalytic action of thiamin in the anaerobic pyruvate metabolism of the propionic acid bacteria and the single species of heterolactic acid bacteria is clearly brought out in table 1. In the absence of thiamin, *Propionibacterium peterssonii* is unable to oxidize pyruvate; on the addition of 2 micrograms of the vitamin to 2 cc. of the cell suspension, pyruvate is attacked. Similar results are obtained with suspensions of *Propionibacterium pentosaceum* and *Lactobacillus mannitopoeus*. *Escherichia coli* and *Aerobacter indologenes*, grown in a thiamin-free medium, do not show an increase in pyruvate metabolism on the addition of thiamin. They are capable of synthesizing the vitamin probably as rapidly as it is required. Sunderlin and Werkman (3) in 1928 reported the synthesis of vitamin B by *E. coli*.

Diphosphothiamin or cocarboxylase is essential for the decarboxylation of pyruvic acid by yeast. Dried yeast can be freed of its cocarboxylase by washing with alkaline phosphate. It now no longer can break down pyruvic acid except on the addition of cocarboxylase; free thiamin is ineffective. Since we had found that free thiamin stimulates the propionic and lactic acid bacteria, it was of interest to determine whether or not the thiamin was esterified before it catalyzed the breakdown of pyruvic acid. Present evidence indicates that such is the case.

We have found that *P. pentosaceum* is capable of synthesizing cocarboxylase. If *P. pentosaceum* is transferred from a yeast extract-peptone medium to a basal medium free of thiamin, the thiamin content of the cells is almost completely depleted by the third transfer on the basal medium. If to these cells, after thorough washing, free thiamin and phosphate buffer are added, a synthesis of cocarboxylase occurs. This is evidenced by data presented in table 2.

TABLE 2. *Synthesis of cocarboxylase by depleted cells of Propionibacterium*

Activator	Supernate 1	Supernate 2	2.3 micrograms cocarboxylase	Control
mm^3 CO_2 in 30 min.	324	28	236	21

Note: Test cells—yeast washed free of cocarboxylase.
Substrate—sodium pyruvate.

Supernate 1 had been obtained from a mixture of thiamin depleted cells, thiamin and phosphate buffer after 4 hours' incubation at 30° C. Supernate 2 was an identical mixture which was not incubated, but boiled immediately after mixing. As is readily seen, yeast washed free of cocarboxylase is reactivated by that synthesized by the bacteria, leaving little doubt that *P. pentosaceum* is capable of synthesizing cocarboxylase from thiamin and phosphate buffer.

Further evidence that thiamin is converted into cocarboxylase by thiamin deficient propionic acid bacteria may be obtained by a comparison of the stimulating action of free thiamin and crystalline cocarboxylase in the anaerobic pyruvate metabolism of these cells. Such data are presented in table 3.

TABLE 3. *Comparison of vitamin B_1 and cocarboxylase as stimulants in the anaerobic pyruvate metabolism of P. pentosaceum grown in vitamin B_1 deficient media*

Cup	1	2	3	4	5
Activator	None	0.25 microgram vitamin B_1		0.34 microgram cocarboxylase	
		Main vessel	Side cup	Main vessel	Side cup
Interval min. 0–30	28	95	51	95	60
30–60	8	64	35	59	51
60–90	7	54	32	53	50
90–120......	7	55	41	56	52
120–150......	6	42	37	44	50
150–180......	5	46	41	46	47

Note: Results in mm^3 corrected for endogenous CO_2.

In cups 2 and 4, containing equimolar concentrations of thiamin and cocarboxylase, the cells had been incubated with the activators 45 minutes before the addition of pyruvic acid. The rates of gas production during the

intervals recorded are similar. In cups 3 and 5, equimolar quantities of the two activators were added to the cells at the time of the addition of pyruvic acid—no initial period of incubation of the cells and activators was permitted. Cup 5 containing added cocarboxylase attained the rates of the others after a 30 minute lag. Cup 3 containing the free thiamin required from 90 to 120 minutes to attain the rates of the others. It is reasonable to assume that this time is required for the synthesis of the diphosphoric ester of thiamin. In any case, if no previous incubation with the bacterial cells is permitted, cocarboxylase is a more effective stimulant than is free thiamin.

Work by Robbins (4) of the Missouri Botanical Gardens and by others has shown that some fungi are capable of synthesizing either or both of the two fractions[1] of the thiamin molecule to satisfy their growth requirements. We have tested the ability of *P. pentosaceum* to utilize either fraction separately or both together as a substitute for thiamin. It has been found that cells of *P. pentosaceum* cannot use the individual fractions as a substitute for the vitamin even when both are present—apparently these bacteria are unable to couple the two fractions to form the complete vitamin.

In reporting on the nutrition of the propionic acid bacteria, Wood, Andersen, and Werkman (5) have shown that one of them, *P. pentosaceum* can be trained to grow as well in the absence of thiamin as in its presence. Their data indicate that adaptation occurred after continuous serial transfer in an ammonium sulfate medium containing only ether extract of Difco yeast extract as a stimulant; the organism gradually acquired the ability to dispense with the vitamin. These investigators did not determine whether the organism trained to dispense with the vitamin acquires the ability to synthesize it, or whether its metabolism is diverted so that the vitamin is no longer required. We have found that the organism acquires the ability to synthesize thiamin.

TABLE 4. *Comparative yields of cells, their vitamin B_1 content and anaerobic pyruvate metabolism*

	Yield of wet cells cc.	Vitamin B_1 content per gram dry cells micrograms	Anaerobic pyruvate metabolism mm^3 CO_2/hr.
"Trained" cells	1.15	6.25	120.3
"Untrained" cells	0.50	0.40	15.2
Ratio $\frac{\text{"trained"}}{\text{"untrained"}}$	2.3	15.6	7.9

Table 4 summarizes the results obtained in a series of experiments comparing cells of *P. pentosaceum* which had been carried three transfers ("untrained cells") and ten transfers ("trained cells") on a basal medium free of thiamin.

[1] Pyrimidine fraction employed was 2-methyl-5-bromomethyl-6-amino pyrimidine hydrobromide.

Thiazole fraction employed was 4-methyl-5-β-hydroxyethyl thiazole.

LITERATURE CITED

1. Peters, R. A.
1936. Pyruvic acid oxidation in brain. Biochem. J., **30**: 2206.

2. Tatum, E. L., H. G. Wood, and W. H. Peterson
1936. Growth factors for bacteria. Biochem. J., **30**: 1898.

3. Sunderlin, G., and C. H. Werkman
1928. Synthesis of vitamin B by microörganisms. J. Bact., **16**: 17.

4. Robbins, W. J.
1938. Organisms requiring vitamin B_1. Proc. Nat. Acad. of Sciences U. S. A., **24**: 53.

5. Wood, H. G., A. A. Andersen, and C. H. Werkman
1938. Nutrition of the propionic acid bacteria. J. Bact., **36**: 201.

6. Silverman, M., and C. H. Werkman
1938. Vitamin B_1 in bacterial metabolism. Proc. Soc. Exp. Biol. Med., **38**: 823.

7. Silverman, M., and C. H. Werkman
1939. Bacterial synthesis of cocarboxylase. Enzymologia, **5**: 385.

8. Silverman, M., and C. H. Werkman
1939. Bacterial synthesis of cocarboxylase. Proc. Soc. Exp. Biol. and Med., **40**: 369.

9. Silverman, M., and C. H. Werkman
1939. Function of vitamin B_1 in anaerobic bacterial metabolism. Iowa State Coll. J. of Science, **13**: 107.

10. Silverman, M., and C. H. Werkman
1939. Adaptation of the propionic acid bacteria to vitamin B_1 synthesis including a method of assay. J. Bact., **38**: 25.

ASYMPTOTIC SOLUTION OF A BOUNDARY VALUE PROBLEM[1]

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Many problems in the applications of mathematics lead to questions of the following kind: let $L^n(y)$ denote a differential expression—partial or ordinary—of order n, λ a parameter, and $y = y(x,\lambda)$ the solution of a certain boundary value problem concerning an equation of the form

$$L^n(y) + \lambda L^m(y) = 0 \qquad (m < n). \tag{1}$$

The questions are then: first, whether—as seems plausible—$\lim_{\lambda\to\infty} y(x,\lambda)$ exists and is a solution of the equation $L^m = 0$; second, what boundary conditions this limit satisfies since $y(x,\lambda)$ satisfies n boundary conditions while $L^m = 0$ is an equation of order $m < n$.[2]

The investigation of the relationship between the theories of viscous and ideal liquids (vanishing viscosity) offers famous examples for problems of this kind;[3] another example is furnished by the mathematical theory of the skin effect.[4]

In some former papers the present author dealt with certain linear and non-linear problems of the type mentioned above for the case $m = 0$.[5] It is the aim of the present note to give a simple example in which the method used in these papers can be applied also for the case $m = 1$.

Theorem. *Let $y(x,\lambda)$ be the solution of the boundary value problem*

$$\text{(1a)}\quad y'' + \lambda(y' - y) = \lambda f, \qquad\qquad \text{(1b)}\quad y(0) = y(1) = 0$$

where λ is a positive parameter and the given function $f(x)$ is continuous in the interval $0 \leqq x \leqq 1$. Let $\eta(x)$ be the solution of the problem

$$\text{(2a)}\quad \eta' - \eta = f, \qquad\qquad \text{(2b)}\quad \eta(1) = 0.$$

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[1] Presented to the American Mathematical Society, November 26, 1938.

[2] The important investigations of Birkhoff, Tamarkin, Langer, Trjitzinski, and other writers dealing with the asymptotic integration of ordinary differential equations lie in another direction. References concerning these investigations may, for instance, be found in W. J. Trjitzinski's paper, "Theory of linear differential equations containing a parameter," Acta Mathematica, **67**:1-50.

[3] See C. W. Oseen, Hydrodynamik, Leipzig, 1927.

[4] For a treatment from a purely mathematical point of view, see E. Rothe, Zur Integralgleichung des Skineffekts, Journal f. riene u. angewandte Mathematik, **170** (1934), where other references concerning papers on the skin effect may be found.

[5] Über asymptotische Entwicklungen bei Randwertaufgaben elliptischer partieller Differentialgleichungen, Mathematische Annalen, 108 (no. of volume) (1933); Über asymptotische Entwicklungen bei Randwertaufgaben der Gleichung $\Delta\Delta u + \lambda u = \lambda^{\kappa}\psi$, Mathematische Annalen, **109** (1933); Über asymptotische Entwicklungen bei gewissen nichtlinearen Randwertaufgaben, Compositio Mathematica, 3 (1936).

Then

$$\lim_{\lambda\to\infty} y(x,\lambda) = \eta(x) \quad \text{for } 0 < x \leqq 1.\text{[6]} \tag{3}$$

For the proof, we first state the following:

LEMMA I. Let $F(x,\lambda)$ be a given continuous function $(0 \leqq x \leqq 1, 0 \leqq \lambda < \infty)$ and $M(\lambda) = \underset{0\leqq x\leqq 1}{Max} |F(x,\lambda)|$. The solution v of

$$v'' + \lambda(v' - v) = F \qquad v(0) = v(1) = 0 \tag{4}$$

satisfies the inequality

$$|v(x,\lambda)| < \frac{5M(\lambda)}{\lambda} \tag{5}$$

for sufficiently large λ.

Proof of Lemma I. Putting

$$v = e^{-\frac{1}{2}\lambda x} z, \tag{6}$$

one gets from (4)

$$z'' - \nu z = F\, e^{\frac{1}{2}\lambda x} \qquad z(0) = z(1) = 0 \tag{7}$$

with

$$\nu = \frac{\lambda^2}{4} + \lambda. \tag{8}$$

The solution of (7) may be written in the form

$$z(x) = \int_0^1 g(x,\xi,\lambda)\; F(\xi,\lambda)\; e^{\frac{1}{2}\lambda\xi}\, d\xi \tag{9}$$

where g is the Green's function belonging to (7), namely,

$$g(x,\xi,\lambda) = \frac{-1}{\sqrt{\nu}\,\sinh\sqrt{\nu}} \begin{cases} \sinh\sqrt{\nu}(1-\xi)\sinh\sqrt{\nu}\,x & \text{for } \xi \geqq x \\ \sinh\sqrt{\nu}(1-x)\sinh\sqrt{\nu}\,\xi & \text{for } \xi < x. \end{cases} \tag{9a}$$

We see from (9) and (6) that

$$|v(x)| \leqq M(\lambda) I(x) \tag{10}$$

if

$$I(x) = -\int_0^1 g(x,\xi,\lambda)\; e^{-\frac{1}{2}\lambda(x-\xi)}\, d\xi, \quad M(\lambda) = \underset{0\leqq x\leqq 1}{Max} |F(x,\lambda)|$$

On the other hand, elementary integration furnishes

$$\lambda I(x) = 1 - \frac{e^{-(\sqrt{\nu}+\frac{1}{2}\lambda)x}(1 - e^{-2\sqrt{\nu}(1-x)}) + e^{-(\sqrt{\nu}-\frac{1}{2}\lambda)(1-x)}(1 - e^{-2\sqrt{\nu}x})}{1 - e^{-2\sqrt{\nu}}}.$$

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[6] As $\lambda \to \infty$, the solution of $y'' - \lambda(y' + y) = \lambda f$ for which $y(0) = y(1) = 0$ approaches, in $0 \leqq x < 1$, that solution of $-(\eta' + \eta) = f$ which is zero for $x = 0$. This case may be reduced to the one treated in the above theorem by substituting $1 - x$ for x.

Therefore,

$$\lambda I \leqq 1 + \frac{2}{1 - e^{-2\sqrt{\nu}}}$$

which, on account of (10), proves (5).

LEMMA II. Let $h(x)$ be defined and continuous in $(0, 1)$, u the solution of

(11) $$u'' + \lambda(u' - u) = \lambda h \text{ with } u(0) = u(1) = 0,$$

and w the solution of

(12) $$w' - w = h, \text{ with } w(1) = 0.$$

If then h is such that

(13) $$\int_0^1 h(\xi)\, e^{-\xi}\, d\xi = 0,$$

then

(14) $$\lim_{\lambda \to \infty} u(x) = w(x)$$

uniformly in $0 \leqq x \leqq 1$.

Proof of Lemma II. As is easily seen, the additional condition (13) together with (12) implies $w(0) = 0$. Hence, it follows from (11) and (12) that $v = u - w$ is the solution of problem (4) with $F = -w''$, and (14) is a consequence of (5).

We turn now to the proof of equation (3) of the theorem. If ε is an arbitrary positive number less than 1, it will be sufficient to prove (3) for $\varepsilon \leqq x \leqq 1$. For this purpose we define a function h by

(15) $$\begin{cases} h(x) = f(x) & \text{for } \tfrac{1}{2}\varepsilon \leqq x \leqq 1 \\ h(x) = [f(\tfrac{1}{2}\varepsilon) + (x - \tfrac{1}{2}\varepsilon)\, a]\, e^{x - \frac{1}{2}\varepsilon} & \text{for } 0 \leqq x < \tfrac{1}{2}\varepsilon \end{cases}$$

where

$$a = \frac{4}{\varepsilon} f(\tfrac{1}{2}\varepsilon) + \frac{8}{\varepsilon^2} e^{\frac{1}{2}\varepsilon} \int_{\frac{1}{2}\varepsilon}^1 f(\xi)\, e^{-\xi}\, d\xi.$$

As may be easily verified, h satisfies the hypothesis (13) of Lemma II, so that (14) is true. On the other hand, it follows from (1) and (11) that $v = y - u$ is the solution of (4) with $F = \lambda(f - h)$. Hence it follows from (9), (6), and the definition of h that

$$y - u = \lambda \int_0^{\frac{1}{2}\varepsilon} [f(\xi) - h(\xi)]\, e^{-\frac{1}{2}\lambda(x-\xi)}\, g(x, \xi, \lambda)\, d\xi.$$

Therefore

(16) $$|\, y - u \,| \leqq -\lambda \int_0^{\frac{1}{2}\varepsilon} e^{-\frac{1}{2}\lambda(x-\xi)} g(x, \xi, \lambda)\, d\xi \cdot \{Max\,|f| + Max\,|h|\}$$

Using the fact that $x > \xi$, one obtains from the definition (9a) of g

$$(17)\quad -\lambda\int_0^{\frac{1}{2}\epsilon} e^{-\frac{1}{2}\lambda(x-\xi)} g(x,\xi,\lambda)\,d\xi = \frac{\lambda}{\sqrt{\nu}}\int_0^{\frac{1}{2}\epsilon} e^{-\frac{1}{2}\lambda(x-\xi)} \frac{\sinh\sqrt{\nu}(1-x)\sinh\sqrt{\nu}\xi\,d\xi}{\sinh\sqrt{\nu}}$$

Since $x-\xi \geqq \frac{1}{2}\varepsilon$, we see from (17) and (16) that $\lim\limits_{\lambda\to\infty}(y-u)=0$. Hence, on account of (14), $\lim\limits_{\lambda\to\infty} y = w$, where w is the solution of the problem (11). But, on account of the definition of h, the solution of problem (11) is identical with the solution η of problem (2a), (2b) for $\frac{1}{2}\varepsilon \leqq x \leqq 1$. This proves equation (3) for $\varepsilon \leqq x \leqq 1$.

THE THEORY OF TOPOLOGICAL ORDER IN SOME LINEAR TOPOLOGICAL SPACES[1]

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1. INTRODUCTION

Let E be a space of the following properties:

1. E is topological, that is, to each subset $A \subset E$ exists a subset $\bar{A} \subset E$, the closure of A, with the following properties. (a) $\overline{(\bar{A})} = \bar{A}$; (b) $\overline{A + B} = \bar{A} + \bar{B}$; (c) if A contains not more than one point, then $\bar{A} = A$.[2]
2. E is a linear vector space.[3] The operations implied in the definition of a linear vector space (addition of points and multiplication of a point with a real number) are supposed to be continuous.
3. There exists at least one convex[4] neighborhood U of the zero point $\mathfrak{o}$ of E which is "bounded," that is, has the following property: if $\alpha_1, \alpha_2 \ldots$ is a sequence of real numbers converging to zero and $\mathfrak{x}_1, \mathfrak{x}_2, \ldots$ an arbitrary sequence of points of U, then $\lim\limits_{n \to \infty} \alpha_n x_n = \mathfrak{o}$.[5]

Throughout this paper we denote by V a closed convex bounded set in E containing at least one interior point and by S its boundary. Let $m < M$ be two positive numbers, $\lambda(\mathfrak{x})$ a continuous function defined for $\mathfrak{x} \subset S$, and $\mathfrak{F}(\mathfrak{x})$ a completely continuous representation of S;[6] the image is supposed to lie in E. We consider the representation

$$\mathfrak{y} = \mathfrak{f}(\mathfrak{x}) = \lambda(\mathfrak{x})\mathfrak{x} + \mathfrak{F}(\mathfrak{x}) \qquad (0 < m \leqq \lambda(\mathfrak{x}) \leqq M). \tag{1.1}$$

In generalization and extension of a former paper,[7] it is the aim of the present paper to develop the theory of the order $u(\mathfrak{f}, S, \mathfrak{y}_0)$ of a point $\mathfrak{y}_0 \subset E$ not lying on the image $\mathfrak{f}(S)$ of S.[8]

In Section 2 a number of properties of the order which are well

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[1] Parts of this paper have been presented to the American Mathematical Society December 28, 1937, and April 8, 1938.

[2] The definition adopted here is the one used in [1] and [5]. (The numbers in brackets refer to the list of references at the end of this paper.) In the terminology used in [2], p. 37 and 60, this is a topological T_1 space.

[3] [3], p. 26.

[4] A subset V of E is said to be convex if $\mathfrak{x}_1 \subset V$, $\mathfrak{x}_2 \subset V$ implies $t\mathfrak{x}_1 + (1-t)\mathfrak{x}_2 \subset V$ for $0 \leqq t \leqq 1$. This set of points is called the segment connecting $\mathfrak{x}_1$ and $\mathfrak{x}_2$.

[5] This definition is due to Kolmogoroff [5].

[6] A representation is said to be completely continuous if it is continuous and if the image of each bounded set is compact.

[7] [10].

[8] For the theory of the order in n-dimensional spaces see for instance [2], Chapter XII.

known for n-dimensional spaces are proved in the special case that E is a normed space.[9]

According to a theorem due to Kolmogoroff[10] each space E can be normed. For the definition of the order in the general case it is, therefore, sufficient to show that the order is independent of the special norm which has been introduced. This is done in Section 3.

In Section 4 we deal with a space E which is normed and strictly convex.[11] In this case it is proved that the equality of the two orders of a point $\mathfrak{y}_0 \subset E$ with respect to two representations of the form (1.1) is not only necessary but also sufficient for the homotopy of the two representations in $E - \mathfrak{y}_0$. Furthermore, the notion of the degree of the representation of an S into an S_1 is introduced. It is proved that this degree is zero if not all points of S_1 are image points. Also, it is shown from the theorem on homotopy just mentioned that the equality of the two degrees of two representations is necessary and sufficient for the homotopy of the two representations in S_1.

2. NORMED SPACES

Throughout this section E is supposed to be a normed linear vector space.[12] An n-dimensional linear subspace of E is denoted by E^n. The representation

$$\mathfrak{s}(\mathfrak{x}) = \mathfrak{x} + \mathfrak{S}(\mathfrak{x}), \tag{2.1}$$

defined in a certain subset of E is called a "layer representation" (with respect to E^n)[13] if $\mathfrak{S}(\mathfrak{x}) \subset E^n$ and if, moreover, $\mathfrak{S}$ is continuous. Evidently a layer representation with respect to E^n is also a layer representation with respect to any E^m which contains E^n.

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[9] E is said to be normed if to each element $\mathfrak{x} \subset E$ is associated a non-negative number $||\mathfrak{x}||$, the norm of $\mathfrak{x}$, of the following properties: $||\mathfrak{x}|| = 0$ only for the zero element of E; $||\lambda\mathfrak{x}|| = |\lambda|\,||\mathfrak{x}||$ for any real number λ; $||\mathfrak{x} + \mathfrak{y}|| \leqq ||\mathfrak{x}|| + ||\mathfrak{y}||$. For two elements $\mathfrak{x}$ and $\mathfrak{y}$ the distance is defined as the norm of the difference. Cf. [3], p. 53. Section 2 is closely related to § 2 of paper [10]; all developments, however, are given in such a way that the present paper may be understood without knowledge of [10] and references to this paper are only made for the proof of some special facts. For readers familiar with [10] I indicate some of the differences between Section 2 of the present paper and § 2 of [10]: the use of boundaries of convex sets instead of spheres, which was necessary in view of Section 3; the introduction of representations of the form (1.1), whereas in [10]—as well as in the fundamental paper [7]—only representations with $\lambda(\mathfrak{x}) \equiv 1$ have been considered (this generalization makes the use of central projection possible); the change in the definition of homotopy (cf. Definitions 4, 5, and Lemma 3 of the present paper.).

[10] [5].

[11] According to a definition due to J. A. Clarkson [4], p. 404, a normed space is called strictly convex if, in case the elements $\mathfrak{x}$ and $\mathfrak{y}$ are both different from the zero element, the equality sign in the inequality of Footnote 9 holds then, and only then, when, for a suitable positive number c, $\mathfrak{x} = c\mathfrak{y}$.

[12] Evidently any normed linear vector space satisfies Conditions 1-3 of the introduction if the closure is defined in terms of the distance in the usual way.

[13] Called "transformation dégénérée" in [6], p. 141, and "Schichtenabbildung" in [10], § 1, 7.

DEFINITION 1. *Let V be a convex bounded set in E containing at least one interior point, S its boundary, $\mathfrak{s}(\mathfrak{x})$ a layer representation defined on S, and $\mathfrak{y}_0$ a point of E not lying on $\mathfrak{s}(S)$. Let E^n be a linear subspace of E possessing the following properties:* (a) *$\mathfrak{s}$ is a layer representation with respect to E^n;* (b) *E^n contains at least one interior point of V;* (c) *E^n contains $\mathfrak{y}_0$. Let V^n be the intersection of V and E^n, and S^{n-1} its boundary. S^{n-1} is then the intersection of E^n and S. The representation $\mathfrak{s}^n(x)$ defined by $\mathfrak{s}^n(\mathfrak{x}) = \mathfrak{s}(\mathfrak{x})$ for $\mathfrak{x} \subset S^{n-1}$ is then a continuous representation of S^{n-1} in the space E^n since $\mathfrak{s}$ is a layer representation with respect to E^n. As $\mathfrak{y}_0$ does not lie on $\mathfrak{s}^n(S^{n-1})$, the order $u(\mathfrak{s}^n, S^{n-1}, \mathfrak{y}_0)$ of $\mathfrak{y}_0$ with respect to $\mathfrak{s}^n(S^{n-1})$ is well defined according to the definition given in the topology of n-dimensional spaces. It can be proved that this order u is the same for all linear subspaces E^n having the properties* (a), (b), *and* (c).[14] *Therefore, it is legitimate to define the order $u(\mathfrak{s}, S, \mathfrak{y}_0)$ of $\mathfrak{y}_0$ with respect to $\mathfrak{s}(S)$ by the equation $u(\mathfrak{s}, S, \mathfrak{y}_0) = u(\mathfrak{s}^n, S^{n-1}, \mathfrak{y}_0)$.*

DEFINITION 2. *Let $\mathfrak{f}(\mathfrak{x})$ be a representation* (1.1) *defined on S with $\lambda \equiv 1$. Let $\mathfrak{y}_0$ be a point not lying on $\mathfrak{f}(S)$ and ε its positive distance from $\mathfrak{f}(S)$. Let $\mathfrak{s}(\mathfrak{x})$ be a layer representation defined on S for which*

$$||\mathfrak{f}(\mathfrak{x}) - \mathfrak{s}(\mathfrak{x})|| < \varepsilon \qquad (\mathfrak{x} \subset S) \tag{2.2}$$

holds[15] *so that $\mathfrak{y}_0$ is different from $\mathfrak{s}(S)$. It can be proved that the order $u(\mathfrak{s}, S, \mathfrak{y}_0)$ given by Definition 1 is the same for all layer representations which satisfy* (2.2).[16] *By definition, we put $u(\mathfrak{f}, S, \mathfrak{y}_0) = u(\mathfrak{s}, S, \mathfrak{y}_0)$ and call this number the order of $\mathfrak{y}_0$ with respect to the image $\mathfrak{f}(S)$ of S.*

DEFINITION 3. *In order to define the order $u(\mathfrak{f}, S, \mathfrak{y}_0)$ of a point $\mathfrak{y}_0$ not lying on $\mathfrak{f}(S)$ for a general representation* (1.1), *we put*

$$\mathfrak{f}(\mathfrak{x}, \mathfrak{y}_0) = \mathfrak{f}_0(\mathfrak{x}) = \mathfrak{y}_0 + \frac{1}{\lambda}[\mathfrak{f}(\mathfrak{x}) - \mathfrak{y}_0] = \mathfrak{x} + \mathfrak{F}_0(\mathfrak{x}) \tag{2.3}$$

where

$$\mathfrak{F}_0(\mathfrak{x}) = \mathfrak{y}_0\left(1 - \frac{1}{\lambda}\right) + \frac{1}{\lambda}\mathfrak{F}(\mathfrak{x}). \tag{2.4}$$

The assumption that $\mathfrak{y}_0$ is not on $\mathfrak{f}(S)$ implies that $\mathfrak{y}_0$ is not on $\mathfrak{f}_0(S)$. Therefore, $u(\mathfrak{f}_0, S, \mathfrak{y}_0)$ is well defined according to Definition 2. By definition, we put $u(\mathfrak{f}, S, \mathfrak{y}_0) = u(\mathfrak{f}_0, S, \mathfrak{y}_0)$.

THEOREM 1. *The order $u(\mathfrak{f}, S, \mathfrak{y}_0)$, as defined in Definition 3, remains constant when $\mathfrak{y}_0$ varies continuously without lying on $\mathfrak{f}(S)$.*

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[14] We omit the proof since it is essentially the same as the one given in [10], § 2, Hilfssatz 2, in the special case that S is a sphere. (A sphere of center $\mathfrak{o}$ and radius r is defined as the set of all points $\mathfrak{x}$ for which $||\mathfrak{x}|| = r$; the set of all points $\mathfrak{x}$ with $||\mathfrak{x}|| \leqq r$ is called a "full" sphere.)

[15] For the proof of the existence of such a layer representation, see [7], § 1, 7, or [10], p. 51.

[16] We omit the proof which is essentially the same as the one given in [10], § 2, Hilfssatz 4.

Before proving this theorem we state the following two lemmas:

LEMMA 1. *For each t of the interval $0 \leqq t \leqq 1$, let $\mathfrak{s}(\mathfrak{x}, t)$ be a layer representation with respect to the linear subspace E^n, which is supposed to be independent of t. $\mathfrak{s}(\mathfrak{x}, t)$ is supposed to be continuous in $(\mathfrak{x}, t)$ and not to contain $\mathfrak{y}_0$. The order $u(\mathfrak{s}, S, \mathfrak{y}_0)$ is then independent of t.*

We omit the proof, which is nearly the same as the one given in [10] (Hilfssatz 3) in the special case of a sphere.

LEMMA 2. *Let $\mathfrak{s}$ be a layer representation defined on S, and $\mathfrak{y}_0$ a point not lying on $\mathfrak{s}(S)$. Denote by ε the positive distance between $\mathfrak{y}_0$ and $\mathfrak{s}(S)$. Then*

$$u(\mathfrak{s}, S, \mathfrak{y}) = u(\mathfrak{s}, S, \mathfrak{y}_0) \quad \text{for } ||\mathfrak{y} - \mathfrak{y}_0|| < \varepsilon. \tag{2.5}$$

The proof follows immediately from the well known corresponding property of the order in n-dimensional spaces if one considers an E^n possessing the properties (a), (b), (c) referred to in Definition 1, and, moreover, containing the point $\mathfrak{y}$ for which (2.5) is to be proved.

Proof of Theorem 1. If $\mathfrak{y}_0$ is a point not lying on $\mathfrak{f}(S)$, ε its distance from $\mathfrak{f}(S)$, and m, M the positive numbers of (1.1), it will be sufficient to prove

$$u(\mathfrak{f}, S, \mathfrak{y}_0) = u(\mathfrak{f}, S, \mathfrak{y}_1) \tag{2.6}$$

for an arbitrary point $\mathfrak{y}_1$ for which the inequality

$$||\mathfrak{y}_1 - \mathfrak{y}_0|| < \frac{\varepsilon m}{5M_1(1+m)} \qquad [M_1 = Max(M, 1)] \tag{2.7}$$

holds. On account of Definition 3, Equation (2.6) is equivalent to

$$u(\mathfrak{f}_0, S, \mathfrak{y}_0) = u(\mathfrak{f}_1, S, \mathfrak{y}_1) \tag{2.8}$$

if

$$\begin{aligned} \mathfrak{f}_0 &= \mathfrak{y}_0 + \frac{1}{\lambda}(\mathfrak{f} - \mathfrak{y}_0) = \mathfrak{x} + \mathfrak{y}_0\left(1 - \frac{1}{\lambda}\right) + \frac{1}{\lambda}\mathfrak{F}, \\ \mathfrak{f}_1 &= \mathfrak{y}_1 + \frac{1}{\lambda}(\mathfrak{f} - \mathfrak{y}_1) = \mathfrak{x} + \mathfrak{y}_1\left(1 - \frac{1}{\lambda}\right) + \frac{1}{\lambda}\mathfrak{F}. \end{aligned} \tag{2.9}$$

To prove (2.8), let $\mathfrak{s}_0(\mathfrak{x})$ and $\mathfrak{s}_1(\mathfrak{x})$ be two layer representations with[15]

$$||\mathfrak{f}_0(\mathfrak{x}) - \mathfrak{s}_0(\mathfrak{x})|| < \frac{\varepsilon}{5M_1}, \quad ||\mathfrak{f}_1(\mathfrak{x}) - \mathfrak{s}_1(\mathfrak{x})|| < \frac{\varepsilon}{5M_1}. \tag{2.10}$$

We state that then

$$u(\mathfrak{f}_0, S, \mathfrak{y}_0) = u(\mathfrak{s}_0, S, \mathfrak{y}_0), \quad u(\mathfrak{f}_1, S, \mathfrak{y}_1) = u(\mathfrak{s}_1, S, \mathfrak{y}_1). \tag{2.11}$$

We have, in fact, from (2.10), (2.9), and the definition of ε

$$||\mathfrak{f}_0 - \mathfrak{s}_0|| < \frac{\varepsilon}{M_1} < ||\mathfrak{y}_0 - \mathfrak{f}_0(S)||$$

so that the first equation (2.11) follows from Definition 2. As to the second equation (2.11), we have from (2.9) and (2.7):

$$(2.12) \qquad ||\mathfrak{f}_0 - \mathfrak{f}_1|| \leqq ||\mathfrak{y}_0 - \mathfrak{y}_1|| \left(1 + \frac{1}{m}\right) \leqq \frac{\varepsilon}{5M_1},$$

and, by this, from (2.9), (2.7) and (2.10)

$$||\mathfrak{y}_1 - \mathfrak{f}_1|| \geqq ||\mathfrak{y}_0 - \mathfrak{f}_0|| - ||\mathfrak{f}_1 - \mathfrak{f}_0|| - ||\mathfrak{y}_0 - \mathfrak{y}_1|| \geqq \frac{\varepsilon}{M_1} - \frac{2\varepsilon}{5M_1} > ||\mathfrak{f}_1(\mathfrak{x}) - \mathfrak{s}_1(\mathfrak{x})||$$

which proves the second equation (2.11), again on account of Definition 2.

For the proof of (2.8), it will, on account of (2.11), be sufficient to prove

$$(2.13) \qquad u(\mathfrak{s}_0, S, \mathfrak{y}_0) = u(\mathfrak{s}_1, S, \mathfrak{y}_1).$$

We prove first

$$(2.14) \qquad u(\mathfrak{s}_1, S, \mathfrak{y}_1) = u(\mathfrak{s}_1, S, \mathfrak{y}_0).$$

This equation follows from Lemma 2 since, because of (2.9), (2.12), (2.10), and (2.7), the inequality

$$||\mathfrak{s}_1 - \mathfrak{y}_0|| \geqq ||\mathfrak{f}_0 - \mathfrak{y}_0|| - ||\mathfrak{f}_0 - \mathfrak{f}_1|| - ||\mathfrak{f}_1 - \mathfrak{s}_1||$$
$$> \frac{\varepsilon}{M_1} - \frac{\varepsilon}{5M_1} - \frac{\varepsilon}{5M_1} > ||\mathfrak{y}_1 - \mathfrak{y}_0||$$

holds. We now prove

$$(2.15) \qquad u(\mathfrak{s}_1, S, \mathfrak{y}_0) = u(\mathfrak{s}_0, S, \mathfrak{y}_0).$$

This equality follows from Lemma 1, for we have, because of (2.10) and (2.12),

$$||\mathfrak{s}_0 - \mathfrak{s}_1|| \leqq ||\mathfrak{s}_0 - \mathfrak{f}_0|| + ||\mathfrak{f}_0 - \mathfrak{f}_1|| + ||\mathfrak{f}_1 - \mathfrak{s}_1|| < \frac{3\varepsilon}{5M_1},$$

and, from this equation together with (2.9 and (2.10), the inequality

$$||\{\mathfrak{s}_0 + t(\mathfrak{s}_1 - \mathfrak{s}_0)\} - \mathfrak{y}_0|| \geqq ||\mathfrak{s}_0 - \mathfrak{y}_0|| - ||\mathfrak{s}_1 - \mathfrak{s}_0||$$
$$\geqq ||\mathfrak{f}_0 - \mathfrak{y}_0|| - ||\mathfrak{s}_0 - \mathfrak{f}_0|| - ||\mathfrak{s}_1 - \mathfrak{s}_0|| > \frac{\varepsilon}{M_1} - \frac{\varepsilon}{5M_1} - \frac{3\varepsilon}{5M_1} > 0$$

follows for $0 \leqq t \leqq 1$.

Since (2.13) follows from (2.14) and (2.15), our theorem is proved.

Our next aim is to prove the invariance of the order with respect to a continuous change of the representation $\mathfrak{f}$. Before doing so we have to define the exact meaning of a "continuous change."

Definition 4. *Let $\lambda(\mathfrak{x}, t)$ be a function and $\mathfrak{F}(\mathfrak{x}, t)$ a representation, both defined for $0 \leqq t \leqq 1$ and for all $\mathfrak{x}$ of a certain bounded subset A of E. We suppose:*

(a) *λ and $\mathfrak{F}$ are continuous as functions of* $(\mathfrak{x}, t)$;

(b) *$M \geqq \lambda \geqq m > 0$, where the constants m and M are independent of t and $\mathfrak{x}$,*

(c) *the set of all points* $\mathfrak{F}(\mathfrak{x}, t)$ *for* $\mathfrak{x} \subset A$ *and* $0 \leqq t \leqq 1$ *is compact.*

Under these conditions we say, by definition, that the representation

$$\mathfrak{f}(\mathfrak{x}, t) = \lambda(\mathfrak{x}, t)\mathfrak{x} + \mathfrak{F}(\mathfrak{x}, t) \tag{2.16}$$

depends continuously on the parameter t. Moreover, if

$$\mathfrak{f}_1(\mathfrak{x}) = \lambda_1(\mathfrak{x})\mathfrak{x} + \mathfrak{F}_1(\mathfrak{x}), \quad \mathfrak{f}_2(\mathfrak{x}) = \lambda_2(\mathfrak{x})\mathfrak{x} + \mathfrak{F}_2(\mathfrak{x}) \tag{2.17}$$
$$(0 < m \leqq \lambda_i(\mathfrak{x}) \leqq M;\ i = 1, 2)$$

are two representations defined in A with continuous λ_i, *and completely continuous* $\mathfrak{F}_i$, *we say, by definition, that* $\mathfrak{f}_1$ *and* $\mathfrak{f}_2$ *can be continuously transformed into each other or that they are homotopic if there exists a representation* $\mathfrak{f}(\mathfrak{x}, t)$ *depending, in the sense just defined, continuously on the parameter t, for which* $\mathfrak{f}(\mathfrak{x}, 0) = \mathfrak{f}_1(\mathfrak{x})$ *and* $\mathfrak{f}(\mathfrak{x}, 1) = \mathfrak{f}_2(\mathfrak{x})$. *If, moreover,* $\mathfrak{f}(\mathfrak{x}, t)$ *has no point in common with a certain subset E′ of E for all t of the interval* $0 \leqq t \leqq 1$, *we say that* $\mathfrak{f}_1$ *and* $\mathfrak{f}_2$ *are homotopic in* $E - E'$.

Definition 5. *We say that the representation* (2.16) *is uniformly continuous* (*with respect to t*) *if in Definition* 4 *the conditions* (a) *and* (c) *are replaced by the following:*

(a′) *For each t,* λ *is continuous and* $\mathfrak{F}$ *completely continuous in dependence on* $\mathfrak{x}$.

(c′) *As functions of t,* λ *and* $\mathfrak{F}$ *are continuous, and uniformly so with respect to* $\mathfrak{x}$, *that is, to any given positive number* ε *there exists a positive number* δ *which is independent of* $\mathfrak{x}$ *such that* $||t' - t''|| < \delta$ *implies*

$$|\lambda(\mathfrak{x}, t') - \lambda(\mathfrak{x}, t'')| < \varepsilon \text{ and } ||\mathfrak{F}(\mathfrak{x}, t') - \mathfrak{F}(\mathfrak{x}, t'')|| < \varepsilon$$

for $0 \leqq t \leqq 1$ and $\mathfrak{x} \subset A$.[17]

Lemma 3. *A uniformly continuous representation* (*Definition* 5) *is continuous in the sense of Definition* 4.

For the proof, we refer to [9], p. 301, proof of Hilfssatz 2.

Remark. The converse of Lemma 3 is not true. An example of a representation satisfying Definition 4, but not the condition (c′) of Definition 5 is the following: let E be the Hilbert space with co-ordinates $x_1, x_2, \ldots$, A the sphere $\sum_{\nu=1}^{\infty} x_\nu^2 = 1$, and $\mathfrak{y} = \mathfrak{f}(\mathfrak{x}, t)$ the representation given by the equations $y_1 = x_1 + \sum_{\nu=1}^{\infty} x_\nu^2 t^\nu$, $y_2 = x_2$, $y_3 = x_3, \ldots$ $\quad (0 \leqq t \leqq 1)$

Theorem 2. *Let* $\mathfrak{f} = \mathfrak{f}(\mathfrak{x}, t)$ *be a representation defined on the boundary S of the convex bounded set V and depending continuously on the parameter t for* $0 \leqq t \leqq 1$ (*Definition* 4). *Let* $\mathfrak{y}_0$ *be a point different from* $\mathfrak{f}(\mathfrak{x}, t)$ *for all* $\mathfrak{x} \subset S$ *and all t. Then the order* $u(\mathfrak{f}, S, \mathfrak{y}_0)$ *is independent of t.*

Before proving Theorem 2, we state the following:

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[17] In the papers [7] and [10] the meaning of "continuous" is, in the terminology of the present paper, "uniformly continuous."

LEMMA 4. *Let* $\mathfrak{f}(\mathfrak{x}, t)$ *be a representation* (1.1), *with* $\lambda = 1$, *defined in a certain subset* A *of* E, *and depending continuously on* t *(Definition* 4); *let* ε *be a positive constant. Then there exist a linear subset* $E^n \subset E$ *which is independent of* t *and a representation* $\mathfrak{s}(\mathfrak{x}, t) = \mathfrak{x} + \mathfrak{S}(\mathfrak{x}, t)$ *of the following properties:*

(a) $\mathfrak{s}$ *is a layer representation with respect to* E^n,

(b) $\mathfrak{s}$ *is continuous in* $(\mathfrak{x}, t)$,

(c) $\|\mathfrak{s}(\mathfrak{x}, t) - \mathfrak{f}(\mathfrak{x}, t)\| < \varepsilon$.

Proof.[18] From Condition (c) in Definition 4 and the Heine-Borel theorem it follows that the set of points $\mathfrak{F}(\mathfrak{x}, t)$ $(\mathfrak{x} \subset A, 0 \leqq t \leqq 1)$ contains a finite number of points $\mathfrak{y}_1, \mathfrak{y}_2, \ldots, \mathfrak{y}_m$ such that, for each $\mathfrak{x}$ and t, the inequality

$$\|\mathfrak{F}(\mathfrak{x}, t) - \mathfrak{y}_\alpha\| < \varepsilon$$

holds for at least one $\alpha (1 \leqq \alpha \leqq m)$. Let us denote by E^n the smallest linear subset of E containing the points $\mathfrak{y}_1, \mathfrak{y}_2, \ldots, \mathfrak{y}_m$ and put

$$\mu_\nu(\mathfrak{y}) = \begin{cases} \varepsilon - \|\mathfrak{y} - \mathfrak{y}_\nu\| & \text{for } \|\mathfrak{y} - \mathfrak{y}_\nu\| \leqq \varepsilon \\ 0 & \text{for } \|\mathfrak{y} - \mathfrak{y}_\nu\| > \varepsilon. \end{cases}$$

If then

$$\mathfrak{S}(\mathfrak{x}, t) = \frac{\sum_{\nu=1}^{m} \mathfrak{y}_\nu \mu_\nu[\mathfrak{F}(\mathfrak{x}, t)]}{\sum_{\nu=1}^{m} \mu_\nu[\mathfrak{F}(\mathfrak{x}, t)]},$$

one sees easily that $\mathfrak{s}(\mathfrak{x}, t) = \mathfrak{x} + \mathfrak{S}(\mathfrak{x}, t)$ has the properties (a), (b), (c).

Proof of Theorem 2. Since $\mathfrak{y}_0$ is different from $\mathfrak{f}(\mathfrak{x}, t)$ it is, on account of the assumptions made concerning λ and $\mathfrak{F}(\mathfrak{x}, t)$, easy to see that there exists a positive constant ε' such that $\|\mathfrak{f}(\mathfrak{x}, t) - \mathfrak{y}_0\| > \varepsilon'$. Hence the representation $\mathfrak{f}_0$ defined in (2, 3) satisfies the inequality

$$\|\mathfrak{f}_0(\mathfrak{x}, t) - \mathfrak{y}_0\| = \frac{1}{\lambda(\mathfrak{x}, t)} \|\mathfrak{f}(\mathfrak{x}, t) - \mathfrak{y}_0\| > \frac{\varepsilon'}{M}$$

and, if $\varepsilon = \dfrac{\varepsilon'}{M}$, the hypotheses of Lemma 4. Now let $\mathfrak{s}$ be a layer representation of the properties described in Lemma 4. By Definition 2, we have $u(\mathfrak{f}_0, S, \mathfrak{y}_0) = u(\mathfrak{s}, S, \mathfrak{y}_0)$. Hence,

$$u(\mathfrak{f}, S, \mathfrak{y}_0) = u(\mathfrak{s}, S, \mathfrak{y}_0) \tag{2.18}$$

since, by Definition 3, $u(\mathfrak{f}, S, \mathfrak{y}_0) = u(\mathfrak{f}_0, S, \mathfrak{y}_0)$. According to Lemma 1, $u(\mathfrak{s}, S, \mathfrak{y}_0)$ is independent of t; hence Theorem 2 follows from (2.18).

An immediate consequence of Theorem 2 is the following theorem

[18] C.f. the proof given by Leray-Schauder ([7], p. 51, second lemma) in the case of a representation not containing a parameter.

which, in analogy to the n-dimensional case,[19] will be referred to as the theorem of Poincaré-Bohl:

THEOREM 3 (Theorem of Poincaré-Bohl). *Let* $\mathfrak{f}(\mathfrak{x})$ *and* $\mathfrak{g}(\mathfrak{x})$ *be two representations defined on the boundary* S *of a convex bounded set* V[21] *and having the same properties as the representation* (1.1). *Let* $\mathfrak{y}_0$ *be a point which, for each* $\mathfrak{x} \subset S$, *is not on the segment*[4] *connecting the points* $\mathfrak{f}(\mathfrak{x})$ *and* $\mathfrak{g}(\mathfrak{x})$. *Then* $u(\mathfrak{f}, S, \mathfrak{y}_0) = u(\mathfrak{g}, S, \mathfrak{y}_0)$.

For the proof, we have simply to apply Theorem 2 with

$$\mathfrak{f}(\mathfrak{x}, t) = (1-t)\,\mathfrak{f}(\mathfrak{x}) + t\mathfrak{g}(\mathfrak{x}) \qquad (0 \leqq t \leqq 1).$$

As is seen from the fact that

$$||(1-t)\mathfrak{f} + t\mathfrak{g} - \mathfrak{y}_0|| = ||\mathfrak{f} - \mathfrak{y}_0 + t(\mathfrak{g} - \mathfrak{f})|| \geqq ||\mathfrak{f} - \mathfrak{y}_0|| - ||\mathfrak{g} - \mathfrak{f}||$$

which holds for $0 \leqq t \leqq 1$, the following theorem is a special case of Theorem 3:

THEOREM 4 (Theorem of Rouché). *If for the two representations* $\mathfrak{f}$ *and* $\mathfrak{g}$ *having the same properties as the representation* (1.1) *the inequality*

$$||\mathfrak{f} - \mathfrak{g}|| < ||\mathfrak{f} - \mathfrak{y}_0||$$

holds, then $u(\mathfrak{f}, S, \mathfrak{y}_0) = u(\mathfrak{g}, S, \mathfrak{y}_0)$.[22]

Since $u(\mathfrak{x}, S, \mathfrak{y}_0) = 1$, the substitution of $\mathfrak{x}$ for $\mathfrak{f}(\mathfrak{x})$ in Theorem 4 immediately gives us

THEOREM 5. *If, for all* $\mathfrak{x} \subset S$, *the norm of the displacement* $\mathfrak{g} - \mathfrak{x}$ *is less than the distance between* $\mathfrak{y}_0$ *and* $\mathfrak{x}$, *then* $u(\mathfrak{g}, S, \mathfrak{y}_0) = 1$.

THEOREM 6. *Let* $\mathfrak{f}(\mathfrak{x}) = \lambda(\mathfrak{x})\mathfrak{x} + \mathfrak{F}(\mathfrak{x})$ $(0 < m \leqq \lambda(\mathfrak{x}) \leqq M)$ *be defined in all points* $\mathfrak{x}$ *of the convex bounded set* V (*not only on its boundary* S). *As usual,* λ *is supposed to be continuous, and* $\mathfrak{F}$ *completely continuous. Let* $\mathfrak{y}_0$ *be a point which is not image of a point of* V. *Then* $u(\mathfrak{f}, S, \mathfrak{y}_0) = 0$.

Proof. The theorem is true in case $\lambda \equiv 1$[23] and, therefore, for the representation (2.3) occurring in Definition 3. Since the assumption that $\mathfrak{y}_0$ is, for every $\mathfrak{x} \subset V$, different from $\mathfrak{f}(\mathfrak{x})$ implies that $\mathfrak{y}_0$ is different from $\mathfrak{f}_0(\mathfrak{x})$ also, we have $u(\mathfrak{f}_0, S, \mathfrak{y}_0) = 0$. According to Definition 3 our theorem is proved.

THEOREM 7. *Let* $\mathfrak{f}(\mathfrak{x}) = \lambda(\mathfrak{x})\mathfrak{x} + \mathfrak{F}(\mathfrak{x})$ *be a representation* (1.1) *defined on the boundary* S *of* V, *and* $\mathfrak{y}_0$ *a point not lying on* $\mathfrak{f}(S)$. *If there exists a ray* h[24] *issuing from* $\mathfrak{y}_0$ *and not intersecting* $\mathfrak{f}(S)$, *then* $u(\mathfrak{f}, S, \mathfrak{y}_0) = 0$.

Proof. On account of Definition 3, it will be sufficient to prove the theorem for the representation $\mathfrak{f}_0(\mathfrak{x})$ defined in (2.3). Since $\mathfrak{y}_0$ is different

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[19] See, for instance, [2], p. 459.

[20] See Footnote 4.

[21] We recall the convention of the introduction that V contains an interior point.

[22] For the n-dimensional case, see [2], p. 459.

[23] Cf. [10], §2, Satz 3, where this fact is proved in case S is a sphere.

[24] A ray h issuing from $\mathfrak{y}_0$ and containing the point $\mathfrak{y}_1$ is the set of all points $\mathfrak{y}_0 + t(\mathfrak{y}_1 - \mathfrak{y}_0)$ for $0 \leqq t < \infty$. The "opposite" ray is the set of all points $\mathfrak{y}_0 - t(\mathfrak{y}_1 - \mathfrak{y}_0)$.

from $\mathfrak{f}_0(S)$, there exists a full sphere V_0 with center y_0 having no point in common with $\mathfrak{f}_0(S)$. We will now define a representation $\mathfrak{f}_0(\mathfrak{x})$ for all points $\mathfrak{x}$ of V which on S is identical with the given representation $\mathfrak{f}_0(\mathfrak{x})$. For this purpose, let $\mathfrak{x}_1$ be an arbitrary but fixed interior point of V. By definition, we put $\mathfrak{f}_0(\mathfrak{x}_1) = \mathfrak{y}_1$, where $\mathfrak{y}_1$ is an arbitrarily determined point in the interior of V_0, different from $\mathfrak{y}_0$ and on the ray opposite to h.[24] If $\mathfrak{x}$ is a point of V which is different from $\mathfrak{x}_1$, let $\bar{\mathfrak{x}}$ be the intersection of S and of the ray issuing from $\mathfrak{x}_1$ and containing $\mathfrak{x}$. If, then, t is given by the equation $\mathfrak{x} = \mathfrak{x}_1 + t(\bar{\mathfrak{x}} - \mathfrak{x}_1)$, we define $\mathfrak{f}_0(\mathfrak{x}) = \mathfrak{y}_1 + t[\mathfrak{f}_0(\bar{\mathfrak{x}}) - \mathfrak{y}_1]$.[25] From this definition it is easy to see that $\mathfrak{y}_0$ is not an image of a point of V, since the ray issuing from $\mathfrak{y}_1$ and containing $\mathfrak{y}_0$ has no intersection with $\mathfrak{f}_0(S)$. Hence, Theorem 7 follows from Theorem 6.

An immediate consequence of Theorem 1 and Theorem 6 is

THEOREM 8. *Let $\mathfrak{f}$ be a representation satisfying the assumptions of Theorem 6. If $u(\mathfrak{f}, S, \mathfrak{y}_0)$ is defined and different from zero, then there exists a neighborhood of $\mathfrak{y}_0$ consisting entirely of points which are images of points of V.*

A consequence of Theorem 8 and Theorem 5 is

THEOREM 9 (Displacement Theorem). *Let $V, S, \mathfrak{f}$ have the same meaning as in Theorem 8 and $\mathfrak{y}_0$ be an interior point of V. If the norm of the displacement $\mathfrak{f}(\mathfrak{x}) - \mathfrak{x}$ is less than the distance between $\mathfrak{y}_0$ and $\mathfrak{x}$, that is, if $\|\mathfrak{f}(\mathfrak{x}) - \mathfrak{x}\| < \|\mathfrak{y}_0 - \mathfrak{x}\|$, then there exists a neighborhood of $\mathfrak{y}_0$ consisting entirely of image points.*

THEOREM 10 (Fixpoint theorem).[26] *Let $\mathfrak{F}(\mathfrak{x})$ be a completely continuous representation defined in the convex bounded set V.[21] If the image $\mathfrak{F}(S)$ of the boundary S of V lies in V, then there exists at least one fixpoint in V.*

Proof. Let us assume that there is no fixpoint on S, and, under this assumption, prove the existence of a fixpoint in the interior of V, that is, the existence of an interior solution of the equation $\mathfrak{x} = \mathfrak{F}(\mathfrak{x})$, or, what is the same, of the equation

$$\mathfrak{x} + \mathfrak{y}_0 - \mathfrak{F}(\mathfrak{x}) = \mathfrak{y}_0 \tag{2.19}$$

in which $\mathfrak{y}_0$ is an arbitrary but fixed interior point of V. Since $u(\mathfrak{x}, S, \mathfrak{y}_0) = 1$, it follows from Theorem 8 that for the proof of (2.19) it will be sufficient to show that, with $\mathfrak{g}(\mathfrak{x}) = \mathfrak{x} + \mathfrak{y}_0 - \mathfrak{F}(\mathfrak{x})$,

$$u(\mathfrak{g}, S, \mathfrak{y}_0) = u(\mathfrak{x}, S, \mathfrak{y}_0). \tag{2.20}$$

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[25] Since $\mathfrak{f}_0(\mathfrak{x}) = \mathfrak{x} + [(1-t)\quad(\mathfrak{y}_1 - \mathfrak{x}_1) + t\overline{\mathfrak{F}_0}(\mathfrak{x})]$ where $\overline{\mathfrak{F}_0}(\mathfrak{x}) = \mathfrak{F}_0(\bar{\mathfrak{x}})$ for $\mathfrak{x} \neq \mathfrak{x}_1$ and $= 0$ for $\mathfrak{x} = \mathfrak{x}_1$ it will be clear that $\mathfrak{f}_0$ is a representation (1.1) when it is proved that t and $t\mathfrak{F}_0(\bar{\mathfrak{x}})$ depend continuously on $\mathfrak{x}$. This continuity is obvious for $\mathfrak{x} = \mathfrak{x}_1$. For $\mathfrak{x} \neq \mathfrak{x}_1$, see the proof of Lemma 9, Section 4.

[26] For literature concerning fixpoint theorems in function spaces see, for instance, [10], Footnote 12.

As is seen from the theorem of Poincaré-Bohl (Theorem 3), (2.20) will be proved if we can show that the equation

$$\mathfrak{x} + t[\mathfrak{y}_0 - \mathfrak{F}(\mathfrak{x})] = \mathfrak{y}_0 \tag{2.21}$$

has for no t of the interval $0 \leqq t \leqq 1$ a solution $\mathfrak{x}$ which lies on S.

Since $\mathfrak{y}_0$ is an interior point of V, this is obvious for $t = 0$, and since, by assumption, (2.19) has no solution on S, it is also obvious for $t = 1$. Let us now assume that for a certain t-value of the open interval $0 < t < 1$ (2.21) has a solution $\mathfrak{x} \subset S$. Since, then,

$$\mathfrak{F}(\mathfrak{x}) = \mathfrak{y}_0 + \frac{\mathfrak{x} - \mathfrak{y}_0}{t} \qquad (\mathfrak{x} \subset S)$$

the image $\mathfrak{F}(\mathfrak{x})$ of $\mathfrak{x}$ would be in the exterior of V, which is in contradiction with the hypothesis of our theorem that $\mathfrak{F}(\mathfrak{x}) \subset V$ for $\mathfrak{x} \subset S$.

3. GENERAL SPACES

In this section, we denote by E a space having the properties 1, 2, and 3 stated at the beginning of the introduction. The aim of the present section is to extend the notion of the order and the main theorems proved in Section 1 to such a space E.

According to a theorem proved by Kolmogoroff,[27] it is possible to introduce in E a norm $||\mathfrak{x}||$ in such a manner that a point $\mathfrak{a} \subset E$ belongs then and only then to the closure $\overline{A}$ of the set $A \subset E$ if for each positive number ε the full sphere $||\mathfrak{x} - \mathfrak{a}|| \leqq \varepsilon$ contains a point of A.[28] For the sake of brevity, we will call such a norm a consistent norm.

Now let V be a convex bounded set in E containing at least one interior point and S its boundary. Let $\mathfrak{f}(\mathfrak{x})$ be a representation (1.1) with $\lambda \equiv 1$ defined on S. Let $||\mathfrak{x}||$ be a consistent norm introduced in E. Using this norm, we can, according to Section 1, define the order $u(\mathfrak{f}, S, \mathfrak{y}_0)$ for any point $\mathfrak{y}_0 \subset E$ not lying on $\mathfrak{f}(S)$. Then the following lemma holds:

Lemma 5. *The order $u(\mathfrak{f}, S, \mathfrak{y}_0)$ is the same for all consistent norms.* This lemma, the proof of which will be given afterwards, justifies the following:

Definition 6. *Let $\mathfrak{f}(\mathfrak{x})$ be a representation* (1.1) *with $\lambda \equiv 1$ defined on the boundary S of the convex bounded set $V \subset E$ which contains at least one interior point. The order $u(\mathfrak{f}, S, \mathfrak{y}_0)$ of the point $\mathfrak{y}_0$ not lying on $\mathfrak{f}(S)$ with respect to $\mathfrak{f}(S)$ is, by definition, equal to the order obtained by norming E with a consistent norm. For a general representation* (1.1) *the order is then given by Definition* 3.

An immediate consequence of this definition and Lemma 5 is

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[27] [5].

[28] In other words, the topological correspondence given by defining $||\mathfrak{x} - \mathfrak{y}||$ as the distance between $\mathfrak{x}$ and $\mathfrak{y}$ is the same as the original correspondence between $\overline{A}$ and A (see, for instance, [2], p. 28).

THEOREM 11. *The following theorems of Section* 1 *hold for any space* E *satisfying Conditions* 1, 2, *and* 3 *of the introduction: Theorems* 1, 2, 3, 6, 7, 8, *and* 10.

Proof of Lemma 5. For any point $\mathfrak{x} \subset E$, we denote by $||\mathfrak{x}||_i$ $(i = 1, 2)$ two consistent norms of $\mathfrak{x}$, and by $V_i^{\mathfrak{y}}$ a point set which is, with respect to the corresponding norm, a full sphere with center $\mathfrak{y}$. Since $\mathfrak{y}_0$ does not lie on $\mathfrak{f}(S)$, there exists a positive number ε_1 such that the $V_i^{\mathfrak{y}}$ with radius ε_1 contains no point of $\mathfrak{f}(S)$. Since $||\mathfrak{x}||_1$ and $||\mathfrak{x}||_2$ are both consistent norms, the systems of neighborhoods formed by the full spheres with respect to the two norms are topologically equivalent.[29] Hence, there exists a $V_2^{\mathfrak{y}_0}$ with radius $\varepsilon_2 > 0$ such that

$$V_2^{\mathfrak{y}_0} \subset V_1^{\mathfrak{y}_0} \tag{3.1}$$

Now let $\mathfrak{s}$ be a layer representation for which

$$||\mathfrak{f}(\mathfrak{x}) - \mathfrak{s}(\mathfrak{x})||_2 < \varepsilon_2. \tag{3.2}$$

We state that (3.2) implies

$$||\mathfrak{f}(\mathfrak{x}) - \mathfrak{s}(\mathfrak{x})||_1 < \varepsilon_1. \tag{3.3}$$

To show this, we remark that (3.2) and (3.3) are equivalent with

$$\mathfrak{s}(\mathfrak{x}) \subset V_2^{\mathfrak{y}} \tag{3.2a}$$

and

$$\mathfrak{s}(\mathfrak{x}) \subset V_1^{\mathfrak{y}} \tag{3.3a}$$

respectively, if $\mathfrak{y} = \mathfrak{f}(\mathfrak{x})$ and ε_i is the radius of $V_i^{\mathfrak{y}}$. But since the translation $\mathfrak{x}' = \mathfrak{x} + \mathfrak{y} - \mathfrak{y}_0$ transforms $V_i^{\mathfrak{y}_0}$ into $V_i^{\mathfrak{y}}$ $(i = 1, 2)$, (3.1) implies $V_2^{\mathfrak{y}} \subset V_1^{\mathfrak{y}}$. Hence, (3.3a) is indeed a consequence of (3.2a).

Let us now denote by E_i the space E if normed with the norm $||\mathfrak{x}||_i$ and by u_i the order in the normed space E_i. With these notations, it follows from (3.2, (3.3), and Definition 2 in Section 2 that

$$u_1(\mathfrak{f}, S, \mathfrak{y}_0) = u_1(\mathfrak{s}, S, \mathfrak{y}_0), \quad u_2(\mathfrak{f}, S, \mathfrak{y}_0) = u_2(\mathfrak{s}, S, \mathfrak{y}_0). \tag{3.4}$$

We now consider an n-dimensional linear subspace E^n of E which satisfies the conditions (a), (b), and (c) of Definition 1, Section 2. With the notations used in this definition, it is a well known fact in the topology of n-dimensional spaces that the order $u(\mathfrak{s}^n, S^{n-1}, \mathfrak{y}_0)$ of $\mathfrak{y}_0$ with respect to the image $\mathfrak{s}^n(S^{n-1})$ (which is in E^n) is the same whether we consider E^n as normed by the norm $||\mathfrak{x}||_1$ or by the norm $||\mathfrak{x}||_2$. Therefore, it follows from Definition 1 that

$$u_1(\mathfrak{s}, S, \mathfrak{y}_0) = u(\mathfrak{s}^n, S^{n-1}, \mathfrak{y}_0), \quad u_2(\mathfrak{s}, S, \mathfrak{y}_0) = u(\mathfrak{s}^n, S^{n-1}, \mathfrak{y}_0).$$

Hence, on account of (3.4): $u_1(\mathfrak{f}, S, \mathfrak{y}_0) = u_2(\mathfrak{f}, S, \mathfrak{y}_0)$.

[29] See, for instance, [2], p. 31 ("Hausdorffsches Gleichwertigkeitskriterium").

4. STRICTLY CONVEX SPACES

According to the theorem of Kolmogoroff mentioned in Section 3,[30] it is possible to introduce in E a consistent norm. Now we make the assumption that it is possible to introduce such a consistent norm that the space E after being normed is strictly convex.[31] In the present section we will always assume that such a norm has been introduced, and the normed space will again be denoted by E.

Using our usual notations, let V be a convex bounded set containing at least one interior point, S its boundary, and

$$(4.1) \qquad \mathfrak{f}_1(\mathfrak{x}) = \lambda_1(\mathfrak{x})\mathfrak{x} + \mathfrak{F}_1(\mathfrak{x}), \quad \mathfrak{f}_2(\mathfrak{x}) = \lambda_2(\mathfrak{x})\mathfrak{x} + \mathfrak{F}_2(\mathfrak{x})$$

two representations (1.1) defined on S. We then prove

Theorem 12. *Let $\mathfrak{y}_0$ be a point lying on neither $\mathfrak{f}_1(S)$ nor $\mathfrak{f}_2(S)$. The necessary and sufficient condition that $\mathfrak{f}_1$ and $\mathfrak{f}_2$ be homotopic in $E - \mathfrak{y}_0$*[32] *is that*

$$(4.2) \qquad u(\mathfrak{f}_1, S, \mathfrak{y}_0) = u(\mathfrak{f}_2, S, \mathfrak{y}_0)$$

As a preparation for the proof of Theorem 12, we prove the following lemmas.

Lemma 6. *Let E^n be an n-dimensional linear subset of E. To each $\mathfrak{x} \subset E$ corresponds one and only one $\bar{\mathfrak{x}} \subset E^n$ such that for all points $\mathfrak{x}_n \subset E^n$ the inequality*

$$(4.3) \qquad \|\mathfrak{x} - \bar{\mathfrak{x}}\| \leqq \|\mathfrak{x} - \mathfrak{x}_n\|$$

holds.[33]

Proof. From the inequalities $|\,\|\mathfrak{x} - \mathfrak{x}_n'\| - \|\mathfrak{x} - \mathfrak{x}_n''\|\,| \leqq \|\mathfrak{x}_n' - \mathfrak{x}_n''\|$ and $\|\mathfrak{x}_n - \mathfrak{x}\| \geqq \|\mathfrak{x}_n\| - \|\mathfrak{x}\|$ it follows immediately that $\|\mathfrak{x} - \mathfrak{x}_n\|$ is a continuous function of $\mathfrak{x}_n$ and that $\lim\limits_{\|\mathfrak{x}_n\| \to \infty} \|\mathfrak{x} - \mathfrak{x}_n\| = \infty$. Hence, $\|\mathfrak{x} - \mathfrak{x}_n\|$ has a minimum, that is, there exists at least one point $\mathfrak{x}_n = \bar{\mathfrak{x}} \subset E^n$ for which (4.3) holds. It remains to be proved that there exists only one such point $\bar{\mathfrak{x}}$. This is obvious if $\mathfrak{x} \subset E^n$ since then, obviously, $\bar{\mathfrak{x}} = \mathfrak{x}$. Let us now assume that $\mathfrak{x}$ is not in E^n. If then $\bar{\mathfrak{x}}$ is a point satisfying (4.3), and E^{n+1} the $n+1$-dimensional subspace of E which contains E^n and $\mathfrak{x}$, we construct in E^{n+1} the sphere S^n with center $\mathfrak{x}$ and radius $\|\mathfrak{x} - \bar{\mathfrak{x}}\|$. On account of (4.3), E^n is then a plane of support of S^n. But since E^{n+1} is strictly convex, S^n

[30] See Footnote 27.

[31] According to a theorem of Clarkson [4], this assumption is, for instance, fulfilled for any separable Banach space. For the definition of a strictly convex space, see Footnote 11.

[32] Cf. Definition 4, Section 2.

[33] The following example shows that Lemma 6 is not true in a space which is not strictly convex: Let E be a two-dimensional Euclidean plane with rectangular co-ordinates x, y and let $|x| + |y|$ be the norm of the point of co-ordinates (x, y). Let $E^n = E'$ be the straight line $x + y - 1 = 0$, and $\mathfrak{x}$ the point $x = 0$, $y = 0$. The inequality (4.3) is then satisfied for all points $\bar{\mathfrak{x}}$ of E' for which both co-ordinates are non-negative.

has, according to a theorem of Minkowski,[34] exactly one point in common with any supporting plane. For the supporting plane E^n this point is $\bar{\mathfrak{x}}$ which, therefore, is uniquely determined.

Lemma 6 justifies the following:

DEFINITION 7. *The point* $\bar{\mathfrak{x}}$ *uniquely defined by the inequality* (4.3) *is called the projection of* $\mathfrak{x}$ *on* E^n. $d(\mathfrak{x}) = ||\mathfrak{x} - \bar{\mathfrak{x}}||$ *is called the distance between* $\mathfrak{x}$ *and* E^n.

LEMMA 7. *The distance* $d(\mathfrak{x})$ *between* $\mathfrak{x}$ *and its projection* $\bar{\mathfrak{x}}$ *on* E^n *is continuous.*

Proof. Let $\mathfrak{x}$, $\mathfrak{x}_0$ be two points of E, and $\bar{\mathfrak{x}}$, $\bar{\mathfrak{x}}_0$ their projections on E^n. We get from (4.3) with $\mathfrak{x}_n = \bar{\mathfrak{x}}_0$

$$d(\mathfrak{x}) \leqq ||\mathfrak{x} - \bar{\mathfrak{x}}_0|| \leqq ||\mathfrak{x} - \mathfrak{x}_0|| + ||\mathfrak{x}_0 - \bar{\mathfrak{x}}_0|| = ||\mathfrak{x} - \mathfrak{x}_0|| + d(\mathfrak{x}_0).$$

Since the inequality obtained from the preceding one by interchanging $\mathfrak{x}$ and $\mathfrak{x}_0$ also holds, we have $d(\mathfrak{x}_0) - ||\mathfrak{x} - \mathfrak{x}_0|| \leqq d(\mathfrak{x}) \leqq d(\mathfrak{x}_0) + ||\mathfrak{x} - \mathfrak{x}_0||$, which proves the continuity of $d(\mathfrak{x})$.

LEMMA 8. *The projection* $\bar{\mathfrak{x}}$ *of* $\mathfrak{x}$ *on* E^n *depends continuously on* $\mathfrak{x}$.

Proof. Let $\mathfrak{x}_1, \mathfrak{x}_2, \ldots$ be a sequence with $\lim\limits_{\nu \to \infty} \mathfrak{x}_\nu = \mathfrak{x}$; let $\bar{\mathfrak{x}}_\nu$ be the projections of $\mathfrak{x}_\nu$ on E^n, $d = ||\mathfrak{x} - \bar{\mathfrak{x}}||$, and $d_\nu = ||\mathfrak{x}_\nu - \bar{\mathfrak{x}}_\nu||$. It is to be proved that

$$\lim_{\nu \to \infty} \bar{\mathfrak{x}}_\nu = \bar{\mathfrak{x}}. \tag{4.4}$$

Since, on account of Lemma 7, $\lim\limits_{\nu \to \infty} d_\nu = d$, it follows from the inequalities

$$||\bar{\mathfrak{x}}_\nu - \bar{\mathfrak{x}}|| \leqq ||\bar{\mathfrak{x}}_\nu - \mathfrak{x}_\nu|| + ||\mathfrak{x}_\nu - \mathfrak{x}|| + ||\mathfrak{x} - \bar{\mathfrak{x}}|| = d_\nu + ||\mathfrak{x}_\nu - \mathfrak{x}|| + d$$

that the set of all $\bar{\mathfrak{x}}_\nu$ is a bounded subset of E^n. Therefore, the set $\bar{\mathfrak{x}}_\nu$ is compact and for the proof of (4.4) it is sufficient to show that (4.4) holds for any subsequence n_ν for which $\bar{\mathfrak{x}}_{n_\nu}$ converges. If then $\mathfrak{x}' = \lim\limits_{\nu \to \infty} \bar{\mathfrak{x}}_{n_\nu}$, we have

$$||\mathfrak{x} - \mathfrak{x}'|| \leqq ||\mathfrak{x} - \mathfrak{x}_{n_\nu}|| + ||\mathfrak{x}_{n_\nu} - \bar{\mathfrak{x}}_{n_\nu}|| + ||\bar{\mathfrak{x}}_{n_\nu} - \mathfrak{x}'||.$$

Since the first and last terms converge to zero, and, on account of Lemma 7, the second term to d, it follows $||\mathfrak{x} - \mathfrak{x}'|| \leqq d$ which inequality, according to the definition of $\bar{\mathfrak{x}}$ and to Lemma 6, implies $\mathfrak{x}' = \bar{\mathfrak{x}}$, as we wished to prove.

We turn now to the proof of Theorem 12. In view of Theorem 2, Section 2, only the sufficiency of (4.2) has to be proved. If we put

$$\bar{\mathfrak{f}}_i(\mathfrak{x}) = \mathfrak{y}_0 + \frac{1}{\lambda_i(\mathfrak{x})}[\mathfrak{f}_i(\mathfrak{x}) - \mathfrak{y}_0] \qquad (i = 1, 2)$$

we see from Definition 3, Section 2, and the hypothesis (4.2) that

$$u(\bar{\mathfrak{f}}_1, S, \mathfrak{y}_0) = u(\bar{\mathfrak{f}}_2, S, \mathfrak{y}_0). \tag{4.5}$$

Putting

$$\lambda_i(\mathfrak{x}, t) = 1 + t[\lambda_i(\mathfrak{x}) - 1] \qquad (i = 1, 2, \ 0 \leqq t \leqq 1)$$

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[34] [8], pp. 38, 39.

one sees easily that $\mathfrak{f}_1$ and $\bar{\mathfrak{f}}_1$ are homotopic in $E - \mathfrak{y}_0$ as are likewise $\mathfrak{f}_2$ and $\bar{\mathfrak{f}}_2$. It is therefore sufficient to prove that $\bar{\mathfrak{f}}_1$ and $\bar{\mathfrak{f}}_2$ are homotopic in $E - \mathfrak{y}_0$.

Now let ε_1 be the distance between $\mathfrak{y}_0$ and $\mathfrak{f}_1(S)$, and ε a positive number smaller than $\frac{\varepsilon_1}{M}$ and $\frac{\varepsilon_2}{M}$. If then $\mathfrak{g}_i(\mathfrak{x}) = \mathfrak{x} + \mathfrak{G}_i(\mathfrak{x})$ are layer representations with $||\bar{\mathfrak{f}}_i(\mathfrak{x}) - \mathfrak{g}_i(\mathfrak{x})|| < \varepsilon$, we have, on account of (4.5) and Definition 2,

$$u(\mathfrak{g}_1, S, \mathfrak{y}_0) = u(\mathfrak{g}_2, S, \mathfrak{y}_0) \tag{4.6}$$

Putting

$$\bar{\mathfrak{f}}_i(\mathfrak{x}, t) = (1-t)\bar{\mathfrak{f}}_i(\mathfrak{x}) + t\mathfrak{g}_i(\mathfrak{x}),$$

one sees easily that $\mathfrak{f}_1$ and $\mathfrak{g}_1$ are homotopic in $E - \mathfrak{y}_0$, and likewise $\bar{\mathfrak{f}}_2$ and $\mathfrak{g}_2$.

It remains to be proved that $\mathfrak{g}_1$ and $\mathfrak{g}_2$ are homotopic in $E - \mathfrak{y}_0$. To show this, let E^n be a linear subspace of E having the properties (a), (b), and (c) given in Definition 1 (Section 2) with respect to $\mathfrak{g}_1$ as well as to $\mathfrak{g}_2$. Let S^{n-1} denote the intersection of S and E^n, and $\mathfrak{g}_i^n(\mathfrak{x}) = \mathfrak{x} + \mathfrak{G}_i^n(\mathfrak{x}) = \mathfrak{g}_i(\mathfrak{x})$ for $\mathfrak{x} \subset S^{n-1}$ $(i = 1, 2)$. We have then from Definition 1 in connection with (4.6)

$$u(\mathfrak{g}_1^n, S, \mathfrak{y}_0) = u(\mathfrak{g}_2^n, S, \mathfrak{y}_0).$$

Now, it is well known from the topology of n-dimensional spaces that this equality is sufficient (and necessary) for the homotopy of $\mathfrak{g}_1^n$ and $\mathfrak{g}_2^n$ in $E^n - \mathfrak{y}_0$.[35] Therefore, there exists a representation $\mathfrak{g}^n(\mathfrak{x}, t) = \mathfrak{x} + \mathfrak{G}^n(\mathfrak{x}, t)$ defined for $\mathfrak{x} \subset S^{n-1}$ and for $0 \leqq t \leqq 1$, continuous in $(\mathfrak{x}, t)$, such that

$$\mathfrak{g}^n(\mathfrak{x}, 0) = \mathfrak{g}_1^n(\mathfrak{x}), \quad \mathfrak{g}^n(\mathfrak{x}, 1) = \mathfrak{g}_2^n(\mathfrak{x}), \quad \mathfrak{g}^n(\mathfrak{x}, t) \neq \mathfrak{y}_0. \tag{4.7}$$

We now define a representation for all $\mathfrak{x} \subset E^n$ which for $\mathfrak{x} \subset S^{n-1}$ is identical with $\mathfrak{g}^n(\mathfrak{x}, t)$ and denote this extended representation again by $\mathfrak{g}^n(\mathfrak{x}, t) = \mathfrak{x} + \mathfrak{G}^n(\mathfrak{x}, t)$.[36] Let then $\mathfrak{x}$ be a point on S, $\bar{\mathfrak{x}}$ its projection on E^n (Definition 7), and $\mathfrak{g}(\mathfrak{x}, t) = \mathfrak{x} + \mathfrak{G}(\mathfrak{x}, t)$ the representation defined on S and given by

$$\begin{aligned} \mathfrak{G}(\mathfrak{x}, t) = {} & \mathfrak{G}^n(\bar{\mathfrak{x}}, t) + \mathfrak{G}_1(\mathfrak{x}) - \mathfrak{G}^n(\bar{\mathfrak{x}}, 0) \\ & + t\{[\mathfrak{G}_2(\mathfrak{x}) - \mathfrak{G}^n(\bar{\mathfrak{x}}, 1)] - [\mathfrak{G}_1(\mathfrak{x}) - \mathfrak{G}^n(\bar{\mathfrak{x}}, 0)]\} \end{aligned} \tag{4.8}$$

In order to prove the homotopy of $\mathfrak{g}_1$ and $\mathfrak{g}_2$ in $E - \mathfrak{y}_0$, we will show that $\mathfrak{g}(\mathfrak{x}, t)$ possesses the properties required in Definition 4, Section 2. First we have, clearly, $\mathfrak{g}(\mathfrak{x}, 0) = \mathfrak{g}_1(\mathfrak{x})$ and $\mathfrak{g}(\mathfrak{x}, 1) = \mathfrak{g}_2(\mathfrak{x})$. Moreover, it

[35] See, for instance, [2], p. 511, Satz I′, in connection with p. 462, Section 5.

[36] Such a representation may, for instance, be obtained in the following manner: let V^n be the intersection of V and E^n so that S^{n-1} is the boundary of V^n. Let $\mathfrak{x}_0$ be an arbitrary but fixed interior point of V^n. By definition we put $\mathfrak{g}^n(\mathfrak{x}_0, t) = \mathfrak{y}_0$. For any other point $\mathfrak{x} \subset E$ let $\bar{\mathfrak{x}}$ be the uniquely determined (cf. Footnote 39) intersection of S^{n-1} with the ray issuing from $\mathfrak{x}_0$ and containing $\mathfrak{x}$. If then $\mathfrak{x} = \mathfrak{x}_0 + \tau(\bar{\mathfrak{x}} - \mathfrak{x}_0)$, we put $\mathfrak{g}^n(\mathfrak{x}, t) = \mathfrak{y}_0 + \tau[\mathfrak{g}^n(\bar{\mathfrak{x}}, t) - \mathfrak{y}_0]$.

is immediately seen from (4.8) and Lemma 8 that $\mathfrak{S}$ is continuous. Furthermore, the set of all points $\mathfrak{S}(\mathfrak{x}, t)$ $(\mathfrak{x} \subset S,\ 0 \leqq t \leqq 1)$ is compact since this set is a bounded set of E^n.[37] Finally, for $\mathfrak{x} \subset S$, $\mathfrak{y}_0$ is different from $\mathfrak{s}(\mathfrak{x}, t)$. In fact, since $\mathfrak{y}_0$ and $\mathfrak{S}(\mathfrak{x}, t)$ are both points of E^n, this is obvious for a point $\mathfrak{x}$ which does not lie in E^n. But if $\mathfrak{x} \subset E^n$, then $\mathfrak{x} = \bar{\mathfrak{x}}$ and, according to (4.7) and (4.8), $\mathfrak{s}(\mathfrak{x}, t) = \mathfrak{s}^n(\mathfrak{x}, t)$; but $\mathfrak{s}^n(\mathfrak{x}, t)$ is [cf. (4.7)] different from $\mathfrak{y}_0$ since $\mathfrak{x} \subset S^{n-1}$.

We turn now to the notion of the degree $\gamma(\mathfrak{f}, S)$ of a representation (1.1) which is defined on the boundary S of a convex bounded set $V \subset E$ and for which the image $\mathfrak{f}(S)$ lies on the boundary S_1 of a convex bounded set $V_1 \subset E$; each of the sets V and V_1 is supposed to contain at least one interior point. The order $u(\mathfrak{f}, \mathfrak{S}, \mathfrak{y}_0)$ is, according to Theorem 1, Section 2, the same for all interior points $\mathfrak{y}_0$ of V_1. This justifies the following

DEFINITION 8. *The degree* $\gamma(\mathfrak{f}, S)$ *is defined by the equation*

$$\gamma(\mathfrak{f}, S) = u(\mathfrak{f}, S, \mathfrak{y}_0)$$

where $\mathfrak{y}_0$ *is an arbitrary interior point of* V_1.[38]

THEOREM 13. *If* S_1 *contains a point* $\mathfrak{y}_1$ *which is not an image point under the representation* (1.1) *mapping* S *on* S_1, *that is, for which* $\mathfrak{f}(\mathfrak{x}) \neq \mathfrak{y}_1$ *for all* $\mathfrak{x} \subset S$, *then* $\gamma(\mathfrak{f}, S) = 0$.

The proof follows immediately from Theorem 7, Section 2, if one takes as the ray h of this theorem the ray issuing from $\mathfrak{y}_0$ (Definition 8) and containing the point $\mathfrak{y}_1$ of Theorem 13.[39]

Let $\mathfrak{f}_1$ and $\mathfrak{f}_2$ be two representations (1.1) mapping S on S_1. If there exists a representation $\mathfrak{f}(\mathfrak{x}, t)$ satisfying the conditions of Definition 4, Section 2, and for which, moreover, $\mathfrak{f}(\mathfrak{x}, t) \subset S_1$ for $\mathfrak{x} \subset S$ and for $0 \leqq t \leqq 1$, we say that $\mathfrak{f}_1$ and $\mathfrak{f}_2$ are homotopic in S_1. With these notations, we prove

THEOREM 14. *The equality*

$$\gamma(\mathfrak{f}_1, S) = \gamma(\mathfrak{f}_2, S) \tag{4.9}$$

is a neccessary and sufficient condition for the homotopy in S_1 *of* $\mathfrak{f}_1$ *and* $\mathfrak{f}_2$.[40]

Before proving Theorem 14 we state the following

LEMMA 9. *Let* $\mathfrak{y}_0$ *be an interior point of the convex bounded set* $V_1 \subset E$. *For any point* $\mathfrak{y} \subset E$ *different from* $\mathfrak{y}_0$ *let* $\bar{\mathfrak{y}}$ *be the uniquely determined*[39]

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[37] That $\mathfrak{S}(\mathfrak{x}, t) \subset E^n$ follows from (4.8) and from the fact that $\mathfrak{s}_1(\mathfrak{x}, t)$ and $\mathfrak{s}_2(\mathfrak{x}, t)$ are layer representations with respect to E^n. That the set of all $\mathfrak{S}(\mathfrak{x}, t)$ is bounded is a consequence of (4.8) and the fact that the set of all projections $\bar{\mathfrak{x}}$ of the bounded set S is bounded, as is easily seen from the inequality $\|\bar{\mathfrak{x}} - \mathfrak{x}_0\| \leqq \|\bar{\mathfrak{x}} - \mathfrak{x}\| + \|\mathfrak{x} - \mathfrak{x}_0\| \leqq 2\|\mathfrak{x} - \mathfrak{x}_0\|$ where $\mathfrak{x}_0$ is the point defined in the preceding footnote.

[38] Definition 8 is in agreement with the notations used in the topology of n-dimensional spaces.

[39] For a proof that a ray issuing from an interior point $\mathfrak{y}_0$ of V_1 intersects the boundary S_1 of V_1 in exactly one point see the proof given in [2], p. 599, for the n-dimensional case; this proof, however, is, wthout any change, valid also in the case of the space E.

[40] In the case that E is the Hilbert space and S identical with S_1 this theorem has been proved in [9].

intersection of the ray issuing from $\mathfrak{y}_0$ and containing $\mathfrak{y}$ and of the boundary S_1 of V_1. Then $\bar{\mathfrak{y}}$ depends continuously on $\mathfrak{y}$.[41]

Proof of Lemma 9. For $\mathfrak{z} \subset E$, let $t(\mathfrak{z})$ be the real function defined by

$$t(\mathfrak{z}) = \frac{||\mathfrak{z}||}{||\overline{(\mathfrak{y}_0 + \mathfrak{z})} - \mathfrak{y}_0||} \qquad (\mathfrak{z} \neq \mathfrak{o}). \tag{4.10}$$

We have then

$$\bar{\mathfrak{y}} = \mathfrak{y}_0 + \frac{1}{t(\mathfrak{y} - \mathfrak{y}_0)}(\mathfrak{y} - \mathfrak{y}_0). \tag{4.11}$$

In order to prove that $\bar{\mathfrak{y}}$ is continuous in dependence on $\mathfrak{y}$ for $\mathfrak{y} \neq \mathfrak{y}_0$, it will be sufficient to prove that $t(\mathfrak{y} - \mathfrak{y}_0)$ is continuous as function of $\mathfrak{y}$ since, according to (4.10),

$$t(\mathfrak{y} - \mathfrak{y}_0) > 0 \qquad \text{for } \mathfrak{y} \neq \mathfrak{y}_0. \tag{4.12}$$

To prove this continuity of t it will evidently be sufficient to prove that for any two points $\mathfrak{y}_1 \neq \mathfrak{y}_2$ different from $\mathfrak{y}_0$ the inequality

$$t(\mathfrak{y}_2 - \mathfrak{y}_0) \leqq t(\mathfrak{y}_2 - \mathfrak{y}_1) + t(\mathfrak{y}_1 - \mathfrak{y}_0) \tag{4.13}$$

holds since then, on account of (4.10),

$$|t(\mathfrak{y}_2 - \mathfrak{y}_0) - t(\mathfrak{y}_1 - \mathfrak{y}_0)| \leqq t(\mathfrak{y}_2 - \mathfrak{y}_1) \leqq \frac{||\mathfrak{y}_1 - \mathfrak{y}_2||}{\varepsilon}$$

where ε is so small that the full sphere with center $\mathfrak{y}_0$ and radius ε lies in the interior of V_1.

To prove (4.13) we first note that for any positive real number α

$$t[\alpha(\mathfrak{y} - \mathfrak{y}_0)] = \alpha t(\mathfrak{y} - \mathfrak{y}_0) \tag{4.14}$$

since, obviously, $\overline{[\mathfrak{y}_0 + (\mathfrak{y} - \mathfrak{y}_0)]} = \overline{[\mathfrak{y}_0 + \alpha(\mathfrak{y} - \mathfrak{y}_0)]} = \bar{\mathfrak{y}}$.

Let us now assume that (4.13) does not hold for a certain pair of points $\mathfrak{y}_1 \neq \mathfrak{y}_2$ different from $\mathfrak{y}_0$.[42] This assumption would imply the existence of a number τ for which

$$t(\mathfrak{y}_2 - \mathfrak{y}_0) > \tau > t(\mathfrak{y}_2 - \mathfrak{y}_1) + t(\mathfrak{y}_1 - \mathfrak{y}_0). \tag{4.15}$$

We consider then the points $\mathfrak{y}_3$, $\mathfrak{y}_4$, $\mathfrak{y}_5$, and $\mathfrak{y}_6$ defined by

$$\mathfrak{y}_3 = \mathfrak{y}_0 + \frac{1}{\tau}(\mathfrak{y}_1 - \mathfrak{y}_0) \qquad \mathfrak{y}_4 = \mathfrak{y}_3 + \frac{1}{\tau}(\mathfrak{y}_2 - \mathfrak{y}_1)$$

$$\mathfrak{y}_5 = \mathfrak{y}_0 + \frac{(\mathfrak{y}_3 - \mathfrak{y}_0)}{1 - \theta} \qquad \mathfrak{y}_6 = \mathfrak{y}_0 + \frac{\mathfrak{y}_4 - \mathfrak{y}_3}{\theta},$$

where

$$\theta = \frac{t(\mathfrak{y}_4 - \mathfrak{y}_3)}{t(\mathfrak{y}_3 - \mathfrak{y}_0) + t(\mathfrak{y}_4 - \mathfrak{y}_3)}.$$

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[41] Lemma 9 holds in any space E as defined in Section 1.

[42] The ensuing part of the proof follows Minkowski [8], pp. 12, 13, and is reproduced for the convenience of the reader.

From these definitions in connection with (4.15), (4.14), and (4.12) it follows easily that

$$t(\mathfrak{y}_3 - \mathfrak{y}_0) = \frac{1}{\tau} t(\mathfrak{y}_1 - \mathfrak{y}_0) < 1, \qquad t(\mathfrak{y}_4 - \mathfrak{y}_0) = \frac{1}{\tau} t(\mathfrak{y}_2 - \mathfrak{y}_0) > 1,$$

$$t(\mathfrak{y}_5 - \mathfrak{y}_0) = t(\mathfrak{y}_6 - \mathfrak{y}_0) = t(\mathfrak{y}_3 - \mathfrak{y}_0) + t(\mathfrak{y}_4 - \mathfrak{y}_3)$$
$$= \frac{1}{\tau}[t(\mathfrak{y}_1 - \mathfrak{y}_0) + t(\mathfrak{y}_2 - \mathfrak{y}_1)] < 1.$$

Since evidently $t(\mathfrak{y} - \mathfrak{y}_0) < 1$ for an interior and > 1 for an exterior point $\mathfrak{y}$, these inequalities imply

$$\mathfrak{y}_5 \subset V_1, \mathfrak{y}_6 \subset V_1, \mathfrak{y}_4 \not\subset V_1. \tag{4.16}$$

Since V_1 is convex, (4.16) is in contradiction with the fact that $\mathfrak{y}_4 = (1-\theta)\mathfrak{y}_5 + \theta\mathfrak{y}_6$ and $0 < \theta < 1$. Thus (4.13) and, therefore, Lemma 9 are proved.

Proof of Theorem 14. The necessity of the condition (4.8) is clear from Theorem 2, Section 2. To prove that (4.8) is also sufficient, let $\mathfrak{y}_0$ be an arbitrary but fixed interior point of V_1. According to Definition 8, and Theorem 12, $\mathfrak{f}_1$ and $\mathfrak{f}_2$ are then homotopic in $E - \mathfrak{y}_0$. Hence, there exists a representation $\mathfrak{f}(\mathfrak{x}, t)$ defined on S for $0 \leqq t \leqq 1$ which is different from $\mathfrak{y}_0$ and satisfies the conditions of Definition 4, Section 2. If then $\bar{\mathfrak{y}}$ denotes the intersection of S_1 with the ray issuing from $\mathfrak{y}_0$ and containing the point $\mathfrak{y} = \mathfrak{f}(\mathfrak{x}, t)$, it is, on account of Lemma 9, easy to see that

$$\mathfrak{g}(\mathfrak{x}, t) = \frac{||\bar{\mathfrak{y}} - \mathfrak{y}_0||}{||\mathfrak{f}(\mathfrak{x}, t) - \mathfrak{y}_0||}[\mathfrak{f}(\mathfrak{x}, t) - \mathfrak{y}_0]$$

is a representation (1.1) satisfying the conditions of Definition 4, Section 2, for which, moreover, $\mathfrak{g}(\mathfrak{x}, t)$ is always a point of S_1.

REFERENCES

1. Alexandroff, P.
   1916. Über stetige Abbildungen kompakter Räume. Mathematische Annalen, **96**: 555-571.

2. Alexandroff and Hopf
   1935. Topologie, Erster Band. Julius Springer, Berlin.

3. Banach, S.
   1932. Théorie des opérations linéaires. Warszawa.

4. Clarkson, J. A.
   1936. Uniformly convex spaces. Transactions American Mathematical Society, **40**: 396-414.

5. Kolmogoroff
   1934. Zur Normierbarkeit allgemeiner linearer topologischer Räume. Studia Mathematica, **5**: 29.

6. Leray, J.
   1936. Les problèmes non linéaires. L'enseignement mathématique, **35**: 139-151.

7. Leray and Schauder
1934. Topologie et équations fonctionnelles. Annales Scientifiques de l'École Normale Supérieure, **51**: 45-78.

8. Minkowski
1910. Geometrie der Zahlen. Leipzig and Berlin.

9. Rothe, E.
1937. Über Abbildungsklassen von Kugeln des Hilbertschen Raumes. Compositio Mathematica, **4**: 294-307.

10. ———
1937. Zur Theorie der topologischen Ordnung und der Vektorfelder in Banachschen Räumen. Compositio Mathematica, **5**: 177-197.

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